PRE-OPERATIVE DIAGNOSIS AND PROGNOSTIC EVALUATION OF CARCINOMA BREAST-A COMPARATIVE ANALYSIS OF CONVENTIONAL FNAC AND LIQUID BASED CYTOLOGY

DISSERTATION SUBMITTED TO

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI

in partial fulfilment of

the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH – III

TIRUNELVELI MEDICAL COLLEGE

TIRUNELVELI

APRIL-2016

CERTIFICATE

This is to certify that this Dissertation entitled "**PRE-OPERATIVE DIAGNOSIS AND PROGNOSTIC EVALUATION OF CARCINOMA BREAST-A COMPARATIVE ANALYSIS OF CONVENTIONAL FNAC AND LIQUID BASED CYTOLOGY"** is the bonafide original work of **Dr.NANDHITHA.N**, during the period of her Post graduate study from 2013 – 2016, under my guidance and supervision, in the Department of Pathology Tirunelveli Medical College & Hospital, Tirunelveli, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University will be held in April 2016.

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PROTOCOL TITLE: " PRE OPERATIVE DIAGNOSIS AND PROGNOSTIC EVALUATION OF CARCINOMA BREAST. A COMPARATIVE ANALYSIS OF CONVENTIONAL FNAC AND LIQUID BASED CYTOLOGY".

PRINCIPAL INVESTIGATOR: DR. NANDHITHA. N., MBBS.,

DESIGNATION OF PRINCIPAL INVESTIGATOR: RESIDENT IN PATHOLOGY
DEPARTMENT & INSTITUTION: TIRUNELVELI MEDICAL COLLEGE , TIRUNELVELI

Dear , Dr. Nandhitha. N , MBBS,, The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 28.12.2013.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1.	TIREC Application Form	☐
2.	Study Protocol	☐
3.	Department Research Committee Approval	☐
4.	Patient Information Document and Consent Form in English and Vernacular Language	☐
5.	Investigator's Brochure	☐
6.	Proposed Methods for Patient Accrual Proposed	☐
7.	Curriculum Vitae of the Principal Investigator	☐
8.	Insurance /Compensation Policy	☐
9.	Investigator's Agreement with Sponsor	☐
10.	Investigator's Undertaking	☐
11.	DCGI/DGFT approval	☐
12.	Clinical Trial Agreement (CTA)	☐
13.	Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA)	☐
14.	Clinical Trials Registry-India (CTRI) Registration	☐

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

1. The approval is valid for a period of 2 year/s or duration of project whichever is later
2. The date of commencement of study should be informed
3. A written request should be submitted 3weeks before for renewal / extension of the validity
4. An annual status report should be submitted.
5. The TIREC will monitor the study
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7. The PI should report to TIREC within 7 days of the occurrence of the SAE. If the SAE is Death, the Bioethics Cell should receive the SAE reporting form within 24 hours of the occurrence.
8. In the events of any protocol amendments, TIREC must be informed and the amendments should be highlighted in clear terms as follows:
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   f. The amendment is unlikely to be approved by the IEC unless all the above information is provided.
   g. Any deviation/violation/waiver in the protocol must be informed.

STANDS APPROVED UNDER SEAL

Dr.K.Shantaraman MD
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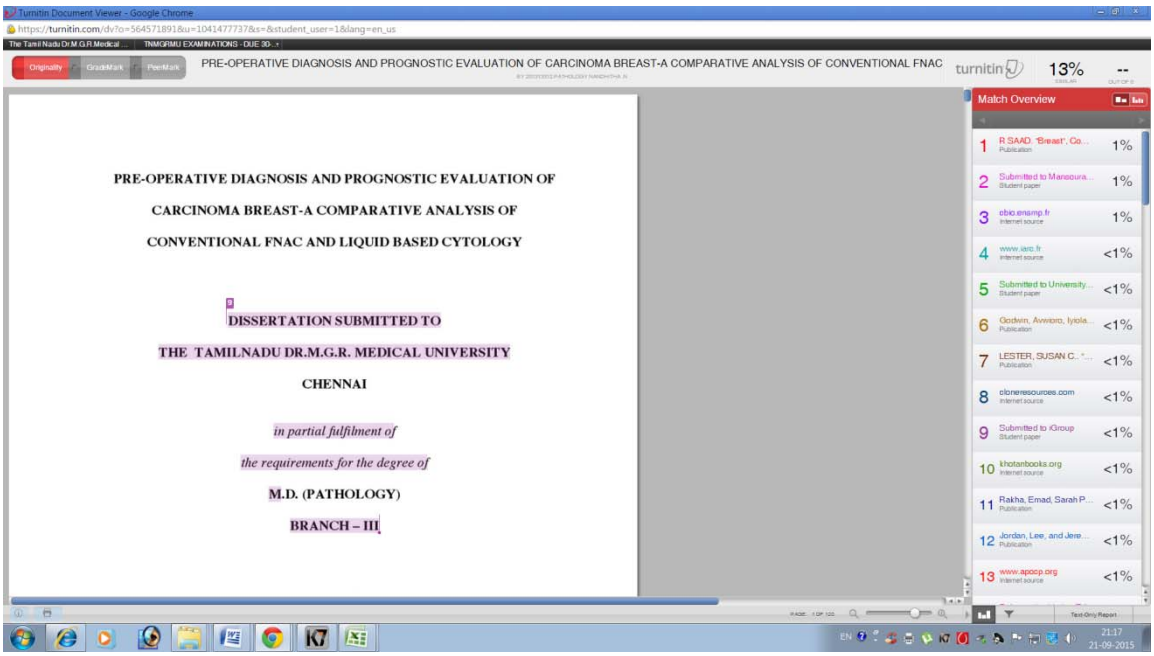
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I solemnly declare that this dissertation titled "**PRE-OPERATIVE DIAGNOSIS AND PROGNOSTIC EVALUATION OF CARCINOMA BREAST-A COMPARATIVE ANALYSIS OF CONVENTIONAL FNAC AND LIQUID BASED CYTOLOGY"** submitted by me for the degree of M.D, is the record work carried out by me during the period of 2013-2016 under the guidance of **Prof. Dr.K.Shantaraman, M.D** Professor of Pathology, Department of Pathology, Tirunelveli Medical College, Tirunelveli. The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, towards the partial fulfilment of requirements for the award of M.D. Degree (Branch III) Pathology examination to be held in April 2016.

Place: Tirunelveli
Date:

DR.NANDHITHA.N,
Department of Pathology,
Tirunelveli Medical College,
Tirunelveli-11

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I consider it my privilege and honour to have worked under the unstinted encouragement, and supervision of **Dr. SITHY ATHIYA MUNAVARAH MD.,** Professor of Pathology.

I thank **Dr. K.Swaminathan MD., Dr.S.Vallimanalan MD, Dr.J.Suresh Durai, MD., Dr.Arasi Rajesh, MD., Dr.Vasuki, MD.,** Professors of Pathology, for their constant support. I also thank the Assistant Professors, for their encouragement. I sincerely thank the Professors and faculties of the Department of General Surgery for providing me the patients for my study. I take this opportunity to thank all my postgraduate colleagues and all the technicians and other members of the Department of Pathology for their kind help.

NANDHITHA.N

ABBREVIATIONS

FNAC	-	Fine Needle Aspiration Cytology
LBC	-	Liquid based cytology
ER	-	Estrogen Receptor
PR	-	Progesterone Receptor
HER2/neu	-	Human Epidermal Growth Factor/neuroblastoma
WHO	-	World Health Organisation
MRI	-	Magnetic Resonance Imaging
ADH	-	Atypical Ductal Hyperplasia
DPX	-	Dibutyl Phthalate Xylene
H&E	-	Hematoxylin and Eosin
CS	-	Conventional smear
NGS	-	Nottingham Grading System
NOS	-	Not otherwise specified
ASCO	-	American Society Of Clinical Oncology
IHC	-	Immunohistochemistry
ICC	-	Immunocytochemistry
PS	-	Proportion score
IS	-	Intensity score
NCT	-	Neo-adjuvant chemotherapy
LABC	-	Locally Advanced Breast Carcinoma
c-DNA	-	Complementary DNA

CK	-	Cytokeratin
EGFR	-	Epidermal growth factor receptor
NST	-	No special type
SERM	-	Selective Estrogen Receptor Modulator
FISH	-	Fluorescent insitu hybridization
PARP	-	Poly ADP Ribose Polymerase
DAB	-	Diamino Benzidine

CONTENTS

ABSTRACT

Background-Fine needle aspiration cytology (FNAC) and Liquid based cytology(LBC) are minimally traumatic techniques used in the pre-operative diagnosis of lesions of breast. They can be used to determine ER,PR and Her-2/neu during the pre-operative period. **Aim of study** - To compare the cytomorphological features of conventional FNAC and Liquid Based Cytology and to evaluate the role of these techniques in the diagnosis as well as in the determination of prognostic and predictive factors. **Materials and methods**-30 cases of FNAC proven malignant lesions of breast were chosen and the cytomorphological features of conventional FNAC were compared with that of Liquid based cytology smears prepared using U-Prep and Nanocyt technology. ER,PR and Her-2/neu was performed on conventional FNAC and LBC smears and the diagnostic and prognostic value of these techniques were evaluated. **Results**-The LBC was found to be better in terms of cellularity, clear background and monolayers while the cell architecture and the cytoplasmic and nuclear details were better preserved in conventional FNAC. Immunocytochemistry on FNAC was found to be very effective in predicting the response of the breast cancer to neoadjuvant chemo or hormone therapy and to determine prognosis. Immunocytochemistry on LBC was not found to be as effective as in conventional smears. The intensity of staining was found to be very poor. **Conclusion**-Conventional FNAC and LBC, though they have their own merits and demerits, they have comparable diagnostic accuracy and a combination of these methods have superior diagnostic value. Immunocytochemistry on conventional FNAC is an effective tool to evaluate the prognostic and predictive factors of breast during the pre-operative period while immunocytochemistry on LBC is not as effective as in LBC.

Key words-Fine needle aspiration cytology, Liquid based cytology, Neoadjuvant chemotherapy

INTRODUCTION

Breast carcinoma is an important cancer affecting women in the industrialized world. Breast cancer is a serious public health issue at present. Breast carcinoma ranks second among prevalence of cancers in women in India[1].

It is estimated that 7% of global burden of breast cancer is borne by India. Breast cancer constitutes one fifths of cancer among women in India[1].The number of deaths in India due to breast cancer is around 50,000 annually[1]. The history of breast cancer in close relatives is a very important risk factor**.** The mutations of the breast cancer genes BRCA1 and BRCA2 is another common risk factor seen in carcinoma breast[2].

FNAC has been accepted by WHO as an important technique in the evaluation of breast cancer[3].It is a safe, cost-effective, minimally traumatic technique for rapid and accurate diagnosis of breast lesions . The efforts to develop specific and scientific methods has led to liquid-based cytology preparation technique. The factors which help LBC to excel routine FNAC are enhanced fixation, paucity of background blood debris, and complete cell transfer. Another added advantage of LBC is that the residual material in the fixative can be used for pre-operative determination of ER,PR and Her-2/neu in breast cancer[4].

There have been only few studies to compare and analyse the cytomorphological features of breast aspirates in conventional smears and liquid based preparations. Also, only a few studies focus on comparison of results of immunocytochemistry in conventional smears and liquid based preparations. Thus

this study was conducted to evaluate the use of conventional FNAC and LBC in pre-operative diagnosis as well as prognosis of breast malignancies.

AIMS AND OBJECTIVES

1. To study the cytomorphology of breast cancer in conventional FNAC and LBC.
2. To study ER, PR and Her-2/neu status of breast malignancy in conventional FNAC and liquid based cytology.
3. To evaluate the diagnostic and prognostic value of conventional smears Vs liquid based cytology.

REVIEW OF LITERATURE

History

It was more than 3,500 years ago that the Ancient Egyptians first identified breast cancer. Cancer was first defined in Egypt at around 1600 BC. Hippocrates described breast cancer as a humoral disease in 460 B.C. The Edwin Smith Papyrus was the one to describe 8 cases of tumors of breast that were treated by cauterization.[5]

In 1757 Henri Le Dran, a French physician proposed that breast cancer can be treated by surgical removal of the tumor along with infected lymph nodes of the armpits .This was also supported by Claude-Nicolas Le Cat. [6]

Embryology of breast

At the fifth week of gestation, the mammary glands arise from the ectodermal mammary ridges. These ridges extend on the ventral surface of the fetus from the axillary to inguinal region bilaterally.However,major part of mammary ridge disappear at about seventh week[7, 8, 9].

The small portion of mammary ridge in the fourth or fifth intercostals space that persist are referred to as primary mammary buds . The underlying mesoderm is penetrated by the primary buds of ectoderm.Mammary lobules are formed from the primary mammary buds that develop into secondary buds by 12th week of gestation[10].

The ectodermal penetration which occurs during fifth month in utero produce 15 –20 radial branching ingrowths into the developing breast. The

lactiferous ducts and their branches are formed from small lumina which develop within the mammary buds . The lactiferous ducts converge to open into a mammary pit , that later transforms into nipple during infancy.[7,8]

Anatomy

Skin and subcutaneous tissue overlie the breast.Breast rests on the pectoral muscle separated by a fascia. The vertical extension of breast is from 2nd to 6th rib and the horizontal extension is from the outer border of sternum to the mid axillary line.The axillary tail of spence is a small extension that extends laterally towards the axilla[11].

The nipple is at the level of 4th intercostal space. Around 15-20 lactiferous ducts pierce the nipple. Areola is a circular pigmented area surrounding the nipple. Areola has numerous modified sebaceous glands. The fibrous strands that pass from the dermis into the breast,called the suspensory ligaments of Cooper[12] anchor the breast to the overlying skin.

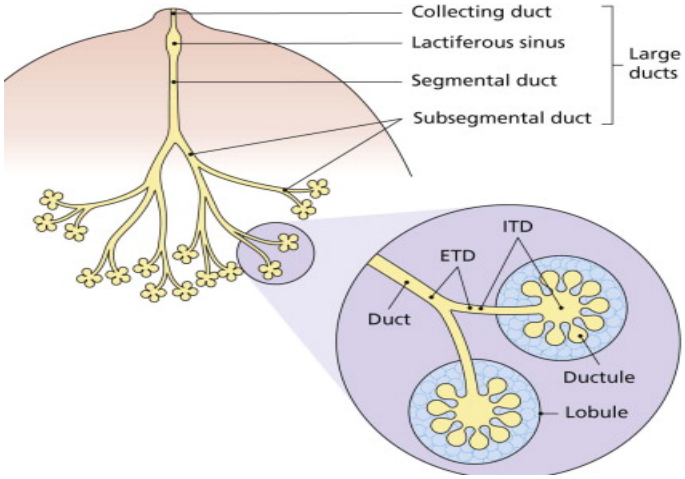

Fig 1: Anatomy of breast

(Collins LC, Schnitt SJ: Breast; In: Mills SE, ed. Histology for pathologists, ed.3. Philadelphia: Lippincott Williams & Wilkins; 2007:57-74.)

The breast parenchyma is made up of glandular tissue which is arranged into lobes[13]. The lobe is made up of terminal duct lobular unit[TDLU] and the large duct system. (fig.1).It is made up of lobule and terminal ductule. Each lobule is a cluster of acini.The sub segmental and segmental ducts connect the TDLU with the lactiferous(collecting) duct. The lactiferous duct opens in to the nipple. The fusiform dilatation that is present between the lactiferous and segmental duct is called the lactiferous sinus [14]

Blood supply

The blood supply of breast is by[15,16],

(i) Internal thoracic artery.

(ii) Axillary artery

(iii) Lateral branches of posterior intercostal arteries.

The venous drainage[16] of the breast is by veins that run along the course of arteries forming an anastomotic circle in the subcutaneous tissue beneath the nipple-areola complex.

From this the veins run as,

1. Superficial veins that drain into internal thoracic vein .

2. Deep veins that drain into internal thoracic, axillary and posterior intercostal veins.[16]

Nerve supply:

Nerve supply is by anterior and lateral cutaneous branches of 4^{th} and 6^{th} intercostal nerves.[16]

Lymphatic drainage

1. Axillary lymph nodes: The anterior group of axillary nodes is the main lymphatic drainage[17] of the breast.The other groups of nodes that receive lymphatic drainage either directly or indirectly are posterior, lateral, central and apical groups of nodes .
2. The internal mammary nodes that are located along internal thoracic vessels.
3. Supraclavicular node, cephalic node, posterior intercostal, subdiaphragmatic and subperitoneal lymph plexus[16]

Lymphatic vessels of breast:

1. The overlying skin of breast except nipple and areola is drained by superficial lymphatics.which drain to the surrounding lymph nodes like axillary, internal mammary, supraclavicular and cephalic node.
2. The parenchyma ,nipple and areola of breast are drained by the deep lymphatics. Axillary nodes drain 75% of lymph, internal mammary nodes drain 20% and the remaining 5 % drain into posterior intercostal nodes.[15]

Histology

The overlying skin of the breast is composed of keratinizing squamous epithelium.This epithelium extends into the orifices of the nipple.This then changes in to a double-layered cuboidal epithelium. The ductal-lobular unit has luminal epithelial cells and basally located myoepithelial cells[18,19]. Luminal cells can be either columnar or cuboidal based on their function. These two cell types originate from the pleuripotent cell in the terminal duct. The glandular epithelial

system rests on a continuous basement membrane. A few scattered endocrine cells may also be found in the normal breast.

Breast has two kinds of stroma-The intralobular stroma and the inter lobular stroma. The intralobular stroma is made up of fibroblast like cells which is hormone responsive, while the interlobular stroma is made up of dense fibrous connective tissue and adipose tissue(fig.2).

The nipple is formed by the lactiferous duct as well as the sebaceous unit. The epidermis of nipple and areola is as same as that of normal skin but has more melanin content in basal layer. There may also be a few clear cells called Toker cells in the basal layer[20].

The luminal cells in the lobules produce milk. Milk ejection during lactation is assisted by the contractile myoepithelial cells. They also produce structural support to lobules.[20]

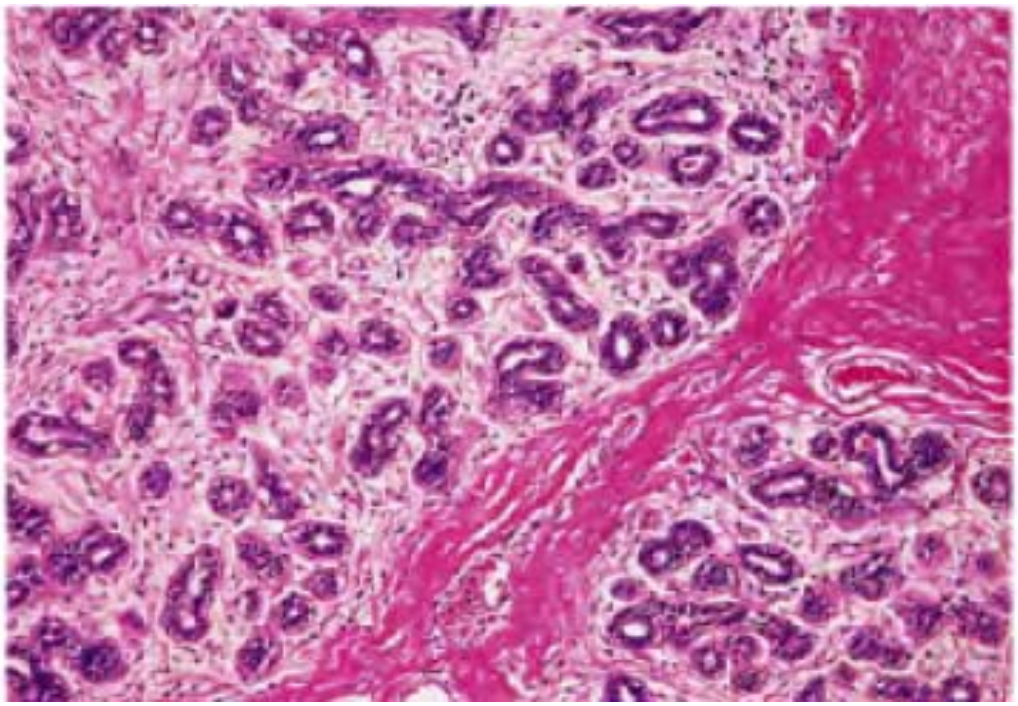

Fig.2. Higher power view - intralobular stroma with interlobular stroma.

Cytokeratin, EMA, lactalbumin and GCDFP-15[21]are positive in the luminal epithelial cells .S-100[22], Smooth Musle Actin, calponin[23], caldesmon(duct portion) and p63(nuclear reactivity) are the markers positive in myoepithelial cells..[24,25]

Physiology of breast

The hormones estrogen and progesterone have a major role in the development of breast[26]. The lobules are relatively inactive during the proliferative phase.After ovulation, the number of acini per lobule[27] increases due to the effect of estrogen and increasing progesterone levels. The intralobular stroma becomes edematous. During menstruation, there will be regression of the lobules with disappearance of the stromal edema due to fall in estrogen and progesterone levels.

The breast becomes completely mature and functional during pregnancy. The number and size of the lobules progressively increase. These lobules are separated by relatively scant stroma. After delivery the luminal cells start producing colostrum which is rich in protein. When the progesterone level begins to drop, in the next 10 days there will be milk secretion which is higher in fat and calories. On stopping lactation the epithelial cells undergo apoptosis, the lobules regress and become atrophic. But however full regression does not occur. During the premenopausal phase, there is involution of the lobules. In elderly females the lobules may become completely atrophic.

FINE NEEDLE ASPIRATION CYTOLOGY OF BREAST MALIGNANCY

The methods which are followed in the investigation of breast lesion that appears suspicious on clinical examination, mammography, sonography, or MRI are

1) Surgical excisional biopsy

2) Core needle biopsy

3) Biopsy by aspiration or fine-needle aspiration (FNA)

FNA is a very useful test when there is low level of suspicion in the diagnosis of malignancy. Another advantage of FNAC is that it can also be used for ancillary tests to study hormone receptor status, to quantify them, to determine, proliferation antigen (e.g., Ki 67), and to perform DNA ploidy analysis and to study gene expression.

The review of literature shows that a French physician, Ku¨n, and a German-Swiss pathologist, Lebert, in 1847 and 1851,described the use of a cannula to obtain cell samples from palpable tumors and used the microscope to identify cancer. In the 1930s [28,29] Martin and Ellis and Stewart used Fine-needle aspiration biopsy of the breast for the first time at Memorial Hospital .

In the 20th century, Hirschfeld was the first person to use a small-caliber needle[30]for the diagnosis of a solid tumor of the skin which got published in 1912 .Fine needle aspiration cytology of tumors has been in use for a very long time.This is a popular procedure because it is cheap[31,32] and is a quick procedure.The risks regarding the procedure is low and it has high diagnostic

accuracy[33,34].The art of performing and interpreting FNA requires expertise and proper training.

It has been proposed by MD Anderson Cancer Center Group that four to six well-visualized cell groups consisting of at least six cells in each cluster and more than ten cells per flat sheet constitute an adequate specimen.[35,36] It is recommended that FNAC should be followed with a biopsy if the FNA findings do not correlate with clinical or radiologic diagnosis of the lesion.[37] The term "Unsatisfactory" smear is used for several reasons like faulty technique, obscuring background debris, poor cellularity etc.

In the present era, breast FNA faces new roles and challenges An accurate diagnosis is expected through FNAC now-a-days .An accurate assessment of the molecular features of tumor and hormone receptor evaluation is also expected in FNAC at present.

The role of FNB in breast lump includes: **1.** the assessment of simple cysts **2.** the diagnosis of suspected recurrence or metastasis in cases of previously diagnosed cancer, **3.** To confirm the diagnosis of inoperable, locally advanced cancer, **4.** the preoperative diagnosis of tumors which appear malignant **5.** To know the diagnosis of any lump which is clinically palpable, either benign or malignant to suggest the line of management, **6.** to obtain tumor cells for advanced studies like DNA analysis, and immunohistochemistry, cell kinetics and molecular studies,7.to diagnose impalpable image-detected lumps which are either benign or malignant[38,]8.To rule out lymph node involvement.9.diagnosis of cystic lesions with suspicious imaging features10.to confirm the diagnosis of

breast cancer in cases where tissue biopsy is not available, not possible or is contraindicated[38].

Fine-needle aspiration (FNA) is a safe, relatively cheap[39] and minimally invasive technique for the diagnosis of breast lesions, The sensitivity of the test increases several fold when it is correlated with clinical history and imaging studies. It is also the least expensive method of diagnosis since it does not require extensive tissue processing. The use of FNA substantially reduces health care expenditure by reducing the number of open biopsies, without compromising early detection.FNA does not need sedation or hospitalization, and it is a very quick procedure. So, It is the most rapid and most versatile method of breast biopsy.

FNAC helps a lot by saving patients from unwanted surgeries and investigations and also helps surgeons to plan quickly and more rationally .The use of FNA of axillary lymph nodes has helped a lot to triage patients for appropriate treatment. Patients who turn out to be positive for metastatic deposits in lymph nodes are subject to axillary dissection or neoadjuvant chemotherapy, whereas those who are negative undergo sentinel lymph node mapping. It is therefore regarded as an inevitable component of the preoperative assessment of pathological processes.

FNAC is also found to be ideal for patients who take anticoagulants and for lesions that lie close to the skin, chest wall, vessels and implant[40]. For superficial, palpable lesions FNAC is a relatively simple procedure and takes very little time in experienced hands[41,42]. The possibility of complications in

FNAC is considerably less. The risk of infection and haematoma formation requiring medical intervention are also extremely rare (0.2%).The risk of pneumothorax also seems to be very less (<0.05%)[43]. There has also been reference in the literature that the incidence of tumour transplantation along the needle track by FNA procedure is only about 0.0045%, and even much lower in superficially located tumours[44]. Another advantage of FNAC is that it is comfortable for aged or frailty patients with comorbidities [45]

Although, a definitive specific diagnosis is not possible by cytology in a number of cases, a differential diagnosis with an estimate of probability can be given which would help to guide the clinician to decide the most efficient further investigations. FNAC also decreases the need for frozen section diagnosis.

However, the utility of breast FNAC depends on the appropriate preparation of cytological conventional smears . Aspirates are best obtained with needles of 23–27 gauge.FNB without aspiration is better for neoplastic breast lesions with increased cell content.

Fig.3-Fine needle aspiration cytology

The disadvantages of FNAC are risks of false positive diagnosis, false negative diagnoses inadequate cellularity, smearing artefacts and abundant back-ground blood debris. Another important factor that determines the utility of FNAC is the expertise of the person performing FNAC and this factor is equally important as sample interpretation to reach the correct diagnosis[46,47,].Another major disadvantage of FNAC is that many breast lesions are heterogeneous, and the small samples obtained with a needle may not be representative even if the procedure is guided by imaging[48].

Multiple passes may overcome this problem, but the number of passes that can be done is restricted inorder to minimize trauma to the patient. The cytological preparations cannot give an idea about the microarchitectural pattern

of the lesion,which is essential in a number of cases to clinch the right diagnosis.Also,the small FNB sample may not be sufficient in many cases to perform ancillary techniques like immune markers. It is essential that the smears are immediately fixed without allowing to dry because drying of smears prior to fixation may distort the morphological features of cells.

One of the major current limitations of FNA biopsy is that it is difficult to differentiate between atypical ductal hyperplasia (ADH) and ductal carcinoma in situ (DCIS) and to differentiate DCIS from invasive carcinoma which has a great influence on the patient's treatment protocol.Another major disadvantage of FNA is it's inability to label a case of low grade carcinoma as malignant lesion.

However, Fine-needle aspiration biopsy of the breast has high degree of accuracy with an average sensitivity of 87% [49,50], specificity of 98-100%, negative predictive value of 87–99%, and efficiency of 89–99%[51]The literature shows that the results of fine needle aspiration cytology are comparable with that of core biopsy[52,53]The accuracy rate of FNA biopsy can be increased if the cytopathologist who performs the FNA biopsy immediately assess the specimen adequacy. The false-negative rate varies from 1 to 31%, with an average rate of 10%.

Grading of breast carcinoma can be done in FNAC using Robinson's grading system(table.1).In this method, six different cytological parameters like cell dissociation, cell size, cell uniformity, nucleolus, nuclear membrane and nuclear chromatin are used to grade the tumors. A low-grade carcinoma is

characterized by tumor cells mostly arranged in clusters ,cells being monomorphic with nuclei being 1-2 times the size of RBCs and nuclei are small and regular with regular chromatin distribution and indistinct nucleolus. A high-grade carcinoma shows polymorphic tumor cells with nuclei of varying size and shape, distinct nucleoli, irregular nuclear membrane, and coarse and irregular chromatin. A score of 1-3 was given to each of these parameters and the tumor was graded by adding up the scores. Tumors that scored in the range of 6-11 were graded I,scores of 12-14 were graded II and scores of 15-18 were graded III.

Table.1.Robinson's grading system for carcinoma breast

	SCORE 1	SCORE 2	SCORE 3
CELL DISSOCIATION	MOSTLY IN CLUSTERS	SINGLE CELLS AND CLUSTERS	MOSTLY IN SINGLE CELLS
NUCLEAR SIZE	1-2 TIMES THE SIZE OF RBC	3-4 TIMES THE SIZE OF RBC	>5 TIMES THE SIZE OF RBC
CELL UNIFORMITY	MONOMORPHIC	MILDLY PLEOMORPHIC	PLEOMORPHIC
NUCLEOLI	INDISTICT/SMALL	NOTICEABLE	ABNORMAL
NUCLEAR MARGIN	SMOOTH	SLIGHTLY IRREGULAR/FOLDS/GROOVES	BUDS AND CLEFTS
CHROMATIN PATTERN	VESICULAR	GRANULAR	CLUMPING AND CLEARING

LIQUID-BASED CYTOLOGY

Liquid Based Cytology is a technique in cytopathology,in which the samples are prepared for examination by collecting samples in a liquid preservative medium before the cells are transferred on to the slide.

Liquid-based preparations excel conventional smears because the cells are better fixed with decreased background debris, and almost complete transfer of cells from the needle hub in to preservative solution occurs. Liquid-based cytology can offer a more definite diagnosis, because the residual material in the solution can be embedded in paraffin blocks and it is possible to get sections with appearance similar to those observed in routine histological sections.LBC reduces false positive and false negative results and optimises the processing flexibility. Increase the cellularity in a defined area and can be used to prepare duplicate and triplicate slides.It can also provide material for the purpose of study of ploidy analysis and immunohistochemistry .[54]Another benefit of LBC is that it is easier and faster to screen and interpret LBPs because the cells are in small areas with clear background.

SurePath (TriPath Imaging, Inc, Burlington, NC), ThinPrep 2000 System (Cytyc Corp, Marlborough, MA), MonoPrep™ (MonoGen, Inc., Lincolnshire,Ill.), which was approved in 2006, are three systems currently approved by the FDA .

In this method, the sample is collected using 23-27G needle in the conventional manner.The material in the hub of the needle is then rinsed into the fixative . This material is allowed to stand for a minimum of 30 minutes and is

then centrifuged for about 10 minutes at 1000 rpm and the supernatant removed. Smears are made from the sediment on a cytospin and stained with haematoxylin and eosin (H&E) by applying Harris haematoxylin for 4 minutes.The smear is then rinsed in water, differentiated in 1%acid-alcohol for 3 seconds, rinsed in water, blued in Scott's solution for 2 minutes and counterstained in 1% eosin for 30 seconds. Smears are then dehydrated through ascending grades of alcohol, cleared in xylene and coverslipped on DPX.

Fig.4.liquid based cytology-cytospin

The quality and cellularity of the samples largely depends on the number of FNA passes and the skills of the practitioner performing the procedure. Compared to conventional FNAC, LBC generally shows presence of small clusters, loss of cohesion and three-dimensional configuration which may lead to an erroneous diagnosis of malignancy. The quality of LBC preparations are similar or slightly superior in comparison to CS.

The cytomorphological features of conventional FNAC and LBC were compared and analysed in various studies based on the parameters like cellularity, background, monolayers, cell architecture and nuclear details and a scoring was given as described in the table given below(table.2 and table.3)

Table.2.Scoring of Cytomorphological features of breast malignancy in FNAC and LBC

Cytologic features	0	1	2	3
cellularity	zero	scanty	adequate	abundant
Back ground debris	zero	occasional	Good amount	abundant
Informative background	absent	Present	-	-
monolayer	absent	occasional	Good amount	-
Cell architecture	Non-recognized	Moderately recognized	Well recognized	-
Nuclear details	poor	fair	good	excellent
Cytoplasmic details	poor	fair	good	excellent

.

Table.3.Comparison of conventional FNAC and LBC

FEATURES	CONVENTIONAL FNAC	LBC
Cellularity	adequate	adequate
Background blood-debris	Good amount	occasional
Informative background	present	absent
Monolayer	occasional	Good amount
Cell architecture	Well recognised	Well recognised
Nuclear details	excellent	good
Cytoplasmic details	excellent	good

Obscuring elements and air-drying and spreading artifacts are significantly reduced in LBC preparations. Although the quality of FNA specimens depends largely on the skills of the practitioner obtaining the samples, the problem of poor smear preparations may be solved by collecting the material in the appropriate vials provided by the different LBC systems.

CARCINOMA BREAST-FINE NEEDLE ASPIRATION CYTOLOGY

DUCTAL CARCINOMA-NOS TYPE

The characteristic features of ductal carcinoma include cellular smears composed of dyscohesive clusters of ductal epithelial cells with cells having scanty cytoplasm and nuclei showing pleomorphism and hyperchromasia. Background shows no bipolar naked nuclei at all.

Using the classification schema based on World Health Organization criteria[54,55] in FNA specimens breast carcinoma can be divided into morphologic subtypes.Among the favorable breast carcinomas are pure mucinous (colloid), true medullary, tubular carcinoma, adenoid cystic carcinoma, papillary carcinoma, and secretory carcinoma. Unfavorable breast malignancies include metaplastic carcinoma, inflammatory carcinoma, pleomorphic lobular carcinoma, and sarcomas

Medullary Carcinoma

Smear studied shows cells arranged in loose syncytial aggregates[56] and as singly scattered cells admixed with bizarre tumor cells with pleomorphic high-grade nuclei,having prominent nucleoli. Background shows numerous lymphocytes and plasma cells[57]

Mucinous (Colloid) Carcinoma

In this subtype,smears studied show aggregates and clusters of uniform tumor cells admixed with occasional signet ring[58] cells in the background of abundant mucin[59,60]

Tubular (Well-Differentiated) Carcinoma

Moderately cellular smears composed of cells arranged in angulated, open rigid tubules with individual cells showing only mild atypia and background shows no bare nuclei.[61,62]

Papillary Carcinoma

Smear studied shows cells arranged in papillaroid clusters , with individual cells showing mild to moderate atypia.[63,64]There are no bipolar nuclei in the background and few hemosiderin laden macrophages are seen.

Micropapillary Carcinoma

In micropapillary carcinoma, smears studied shows lesion composed of cells arranged in tightly cohesive clusters with angulated or scalloped borders with individual cells having naked pleomorphic nuclei seen in the absence of papillary fronds with fibrovascular cores.[65,66].

Secretory Carcinoma

Smear studied shows cells arranged singly and in small clusters with individual cells showing mild atypia .Background shows abundant eosinophilic colloid like material .

Apocrine Carcinoma

Smears studied shows numerous singly scattered cells and cells arranged in syncytial fragments with individual tumor cells having abundant eosinophilic granular cytoplasm and large nuclei with prominent nucleoli.[68].

Lobular Carcinoma

Smears studied from lobular carcinoma shows singly scattered cells and cells arranged in small clusters with individual cells being uniform cells with scanty cytoplasm, central vesicular nuclei with smooth nuclear membrane and inconspicuous nucleoli.[69] Nuclei show budding and moulding. Other features that are common in lobular carcinoma are intracytoplasmic vacuoles, grooving of nuclei and linear arrangement of the cells[70].

Inflammatory Carcinoma

The characteristic feature of this lesion is that grossly, the tumor apperars hyperemic, engorged, and edematous, with peau d'orange skin appearance**.** Smears studied are paucicellular with tumor cells arranged in tight, three-dimensional clusters and individual cells show pleomorphic, hyperchromatic nuclei and increased N/C ratios[71].

Paget's Disease

Paget's disease clinically presents with an eczema-like change of the nipple and areola overlying the breast mass. Smear studied shows scattered tumor cells with abundant pale cytoplasm, pleomorphic, hyperchromatic nuclei in a background showing abundant necrosis, few squamous cells and numerous inflammatory cells.

Metaplastic Carcinoma

Smear studied from metaplastic carcinoma shows mixed population of malignant ductal cells, spindle cells, and multinucleated giant cells . The

undifferentiated spindle cells forms the sarcomatoid component and shows chondrosarcomatous or osteosarcomatous differentiation[72,73].

Squamous Cell Carcinoma of the Breast

Smear studied from squamous cell carcinoma shows sheets of well-differentiated or poorly differentiated squamous cells with increased nuclear cytoplasmic ratio with some of the cells showing intracytoplasmic keratinization of the cells and intercellular bridges and few cells show a tendency to spindle.

CARCINOMA OF BREAST-HISTOPATHOLOGY

Breast cancer is the most common solid epithelial malignant tumor in women.The younger age group shows increase in frequency in carcinoma breast.The incidence of breast carcinoma is around 200 fold more common in women as compared to that in men.Breast cancer can be divided into two principal categories- in situ carcinoma and invasive carcinoma.

DCIS is defined as a proliferation of malignant epithelial cells in parenchymal structures of the breast and is distinguished from invasive carcinoma by the absence of microscopic stromal invasion across the limiting basement membrane.

DCIS can be classified in to low grade and high grade based on nuclear grade.[74] High-grade DCIS refers to those tumors which are negative for hormone receptors and positive for HER-2 and p53.They also exihibit a high proliferation rate,while low-grade DCIS is typically negative for HER-2 and p53 and is positive for hormone receptors and has a low proliferation rate[75].

High Nuclear-Grade Ductal Carcinoma in Situ

Section studied shows lesion composed of large pleomorphic cells with increased nuclear cytoplasmic ratio. The nuclei in this form of the disease are typically more than two and a half to three red blood cells in diameter[76].The chromatin is typically coarse, and large nucleoli are common.Atypical mitoses and necrosis are common. A common feature seen in this type of lesion is a duct filled with solid pattern of tumor cells in the central part of which there is necrosis, known as comedo DCIS.

Low Nuclear-Grade Ductal Carcinoma in Situ

This is composed of evenly spaced cells with small regular nuclei. The nuclei are typically less than two red blood cells in diameter and have indistinct nucleoli.[77].The patterns most commonly seen in this type of lesion are cribriform and micropapillary architecture.The neoplastic cells form geometric punched-out spaces or bulbous projections around which the cells are polarized. Mitoses and necrosis are not common.

Intermediate Nuclear-Grade Ductal Carcinoma in Situ

In intermediate nuclear-grade DCIS, the nuclei show less pleomorphism than in high-grade disease but also lacks the uniformity of the low-grade type.Other features of this tumor are indistinct nucleolus[78],minimal necrosis and cell polarization. The tumor may show solid, cribriform, or micropapillary pattern.

Invasive Carcinoma

Invasive carcinoma of the breast was clinically regarded as a single entity in the past, histologic and molecular analysis have demonstrated that breast cancer is a heterogeneous disease, composed of morphologically and genetically distinct entities with different molecular profiles, behavior, and response to therapy. Clinically, invasive breast cancer is classified according to primary tumor size, lymph node status, and local extent and presence of distant spread. At the morphologic level, breast cancer is classified according to histologic types and grades.

Invasive Carcinoma of No Special Type

Invasive ductal carcinoma of no special type (ductal NST) refers to a group of tumors that do not fall under a specific histologic subtype. Ductal NST is the most common type of invasive carcinoma[79]. The majority of cancers in male breasts are ductal NST.

The carcinoma cells may be arranged in syncytial sheets or cords or be diffusely infiltrative with individual cells having abundant, eosinophilic cytoplasm and nuclei show pleomorphism and hyperchromasia.Stroma may be either cellular or abundant hyalinization may be seen. For a tumor to be typed as ductal NST, it must show the nonspecialized pattern in over 50% of its mass, as judged by thorough examination of representative sections. If the ductal NST pattern comprises between 10% and 49% of the tumor, the rest being of a recognized special type, then it will fall into one of the mixed types[80]. A variant known as pleomorphic carcinoma, is composed of proliferation of pleomorphic

and bizarre tumor giant cells comprising more than 50% of the tumor cells in a background of adenocarcinoma or adenocarcinoma with spindle and squamous differentiation.

Infiltrating Lobular Carcinoma

Infiltrating lobular carcinoma is the second most common type of breast cancer. It is usually associated with older age[81] ,larger tumor size, lower histologic grade, and positive hormone receptors. Infiltrating lobular carcinoma less frequently shows perineural or lymphovascular invasion[82].

The classic subtype accounts for approximately 40% of infiltrating lobular carcinomas. The defining histologic features shared by classic examples are populations of small to moderately sized cells that lack cohesion and are individually dispersed through fibrous tissue or arranged in single files or linear cords that invade the stroma, usually with little host reaction or disturbance to the background tissue architecture[83]. The cords and strands are one or two cells thick; however, broader bands may be seen, and, when prominent, they constitute the trabecular variant of lobular carcinoma. The neoplastic cells are relatively uniform and have round or notched ovoid nuclei with inconspicuous nucleoli and scanty cytoplasm. The nuclei are often eccentrically placed and exhibit little pleomorphism, and mitoses are infrequent.

The majority of infiltrating lobular carcinomas are positive for ER(80%-95%), a rate higher than the 70%-80% observed in ductal NST tumors) and 65% to 75% are positive for PR. Double hormone receptor–positive tumors constitute 70% to 75% of tumors, whereas double negatives are less than 5%. Apart from the

high-grade pleomorphic variant, HER-2 overexpression and proliferation rates are lower than reported in ductal NST. A high proportion of lobular carcinomas express carcinoembryonic antigen, and its intensity tends to correlate with mucin secretion.

Tubular Carcinoma

Tubular carcinoma of the breast is a rare histologic subtype of invasive breast cancer. Tubular carcinoma generally shows favorable prognosis.Tumor size is smaller; has low incidence of lymph node metastases, low incidence of recurrences; and a very favourable overall survival.[84]

The characteristic feature of tubular carcinoma is the presence of open tubules, composed of a single layer of cuboidal cells enclosing a clear lumen. These tubules are generally oval or rounded and often appear angulated and haphazardly arranged . The epithelial cells are small to moderately size and regular, cuboidal, or columnar in shape and contain rounded or oval hyperchromatic low-grade nuclei with little nuclear pleomorphism and inconspicuous nucleoli. Apical snouts are frequent but not pathognomonic. Mitoses are rare. Calcification may be seen in the stroma[85].

For a tumor to be confirmed as tubular it must exhibit a clearly tubular morphology in over 90% of the lesion[86]. If a tumor contains less than 90% tubules, it enters the tubular mixed, ductal, and special type or miscellaneous category. More than 90% of tubular carcinomas are positive for ER and 70% to 80% positive for PR, whereas *HER*-2 amplification is vanishingly rare.

Tubular Mixed Carcinoma

To be included in the tubular mixed category, a tumor must have a stellate configuration with central fibrosis enclosing tubular structures and an infiltrating border of variable thickness composed of cords or sheets of cells with the features of ductal NST carcinoma . Central elastosis is usually present. A minimum cut off point for tubule formation of 50% is now used in routine practice[86].

Invasive Cribriform Carcinoma

Invasive cribriform carcinoma has been applied to this tumor because it exhibits a sieve-like growth pattern similar to that seen in conventional intraductal cribriform carcinoma. It is composed of rounded and angulated masses and islands of small regular epithelial cells embedded in a variable amount of collagenous and reactive-appearing desmoplastic stroma[87]. The nuclei are dense, with little pleomorphism and infrequent mitoses. Within the invasive islands, arches of cells form well-defined punched-outspaces containing variable amounts of mucin-positive secretion and sometimes microcalcifications. For a tumor to be included in the cribriform category this pattern must form at least 90% of the lesion, with the exception that a tumor with 50% or more can be accepted if the rest of the lesion is composed of pure tubular carcinoma.

Mucinous Carcinoma

Microscopically the tumors consist of small islands or clusters of generally uniform, round epithelial cells (10-20 cells) set within extensive lakes of extracellular mucin with mucicarmine-,MUC-2–, and MUC-6–positive content[88]. These mucous lakes are divided by delicate fibrous septa into compartments. The

islands form a trabecular, cribriform, or papillary pattern, sometimes with a tubular arrangement with individual cells being small to medium in size, with minimal amounts of eosinophilic cytoplasm and darkly staining nuclei that exhibit comparatively little nuclear pleomorphism.Pure tumors should comprise entirely of mucinous carcinoma.

Pure mucinous carcinomas consistently express ER (100%) and PR (70%), lack HER-2 expression (97.1%), and show a relatively low level of genetic instability.

Medullary Carcinoma

The epithelial cells are arranged in interconnecting sheets, forming a syncytial network. They are large and pleomorphic with abundant cytoplasm and vesicular nuclei containing one or several nucleoli and showing a proportion of bizarre nuclei and a high mitotic count, that is, histologic grade 3. This pattern should comprise at least 75% of the tumor area with no glandular or tubular component at all.The intervening stroma is scant and contains a moderate to severe lymphoplasmacytic infiltrates and the border of the tumor is pushing rather than infiltrative.Medullary carcinomas typically lack ER and PR expression, as well as *HER-2* amplification (triple negative), and show high proliferative and apoptotic activity.

Atypical medullary carcinoma

Tumors bearing some but not all the features of medullary carcinoma have been designated as atypical medullary carcinoma [89]. A lesser degree of lymphoid infiltrate, microscopic infiltration beyond the main border, or areas of dense

fibrosis may be seen. A tumor may also be classified as atypical medullary if up to 25% is composed of ductal NST and the rest is classic medullary carcinoma.

Invasive Papillary Carcinoma

The characteristic feature is the presence of papillary structures with associated fibrovascular cores. Frank invasion is recognized by the presence of neoplastic cells with infiltrative appearances beyond the zone of reactive stroma and extending into mammary parenchyma and fat. Cytologic appearances are varied, and nuclear pleomorphism and increased numbers of mitoses may be seen[90].

Invasive micropapillary carcinoma

This is applied to an uncommon and unusual variant of invasive breast carcinoma in which epithelial tufts forming micropapillae without a fibrovascular core are located within clear stromal spaces resembling dilated vascular channels[91]. Neoplastic cells show a moderate to marked degree of nuclear pleomorphism and low mitotic activity, and they lack necrosis and a lymphocytic reaction. The neoplastic cells maintain their architectural features in the metastatic sites.[92]

Mixed Types

In mixed ductal and lobular carcinoma are distinct ductal NST and infiltrating lobular elements, the former amounting to between 10% and 90% of the tumor. The mixed ductal and special type of carcinomas includes any tumors composed of a mixture of a special tumor type, such as tubular, invasive

cribriform, or mucinous carcinoma with ductal NST carcinoma in which the latter forms over 10% of the tumor mass.

PROGNOSTIC FACTORS IN CARCINOMA BREAST

They can be divided broadly into two groups, traditional and molecular. The traditional factors can be assessed during conventional examination and histologic evaluation of tumors. Techniques for assessment of molecular markers are less widely available. The outcome for women with breast cancer varies from a normal life expectancy to having only10% chance of being alive in 5 years .This information is important to create awareness in patients about the disease.

Traditional Pathologic Factors

The following pathologic factors, all of which are relatively simple to assess, have been shown to provide clinically useful prognostic information, to a greater or lesser degree.[93,94]

(1)Tumor Size

(2) Lymph Node Status

(3) Histologic Type

(4) Histologic Grade

(5) Lymphovascular Invasion

(6) Necrosis

(7) Stromal Features

(8) patient age

(9)family history

(10)Inflammatory cell infiltrate

(11)apoptosis

(12)angiogenesis

(13)fibrotic foci

(14)perineural invasion

1.Tumor Size

The measured gross size represented by the largest diameter of a mammary carcinoma is one of the most significant prognostic variables. Studies have shown that survival decreases with increasing tumor size and that there is a coincidental rise in the frequency of axillary nodal metastases.[95]

2.Lymph Node status

In the absence of distant metastases ,one of the most important prognostic factors for invasive carcinoma is axillary lymph node status.With no nodal involvement, the 10-year disease-free survival rate is close to 70% to 80%; The 10-year disease free survival decreases significantly with increase in the number of nodes involved.

3.Histologic sub- type

Various special types of invasive carcinomas (tubular, mucinous, medullary, lobular, and papillary) have better disease free survival than NST cancers.

4.Histolgical grading of ductal carcinoma

Grading of breast cancer was first attempted by Green though in 1925.He used about 18 features and it is not popular. In 1993 Haagensen evaluated around 15 histological features to grade carcinoma breast.

The most popular grading system till date was proposed by Bloom in 1950.[92]His grading system was based on three main features which includes degree of tubule formation ,nuclear features and mitotic activity .He classified breast carcinoma into 2 categories –low grade and high grade tumors.

In 1957 this classification was upgraded by modifications of Bloom and Richardson[96]It is also based on degree of tubule formation, nuclear pleomorphism and mitotic activity. But in this classification score of 1 to 3 was given to each criteria according to mild ,moderate or marked degrees. A total score of 3 to 9 was given as follows,

Table 4 : Bloom And Richardson grading system 1957

Score 3-5	Grade 1	Well differentiated tumors
Score 6-7	Grade 2	Moderately differentiated tumors
Score 8-9	Grade 3	Poorly differentiated tumors

Elston- Ellis modification of the Scarff-Bloom-Richardson grading system (Nottingham grading system [NGS])[96]

Tubule Formation

Majority of tumor (>75%) -1 point

Moderate degree (10%-75%)- 2 points

Little or none (<10%) -3 points

Nuclear Pleomorphism

Small, regular uniform cells- 1 point

Moderate increase in size and variability -2 points

Marked variation -3 points

Mitotic Counts

Mitotic count is also graded as 1-3.But it depends on the field diameter used. Mitotic figures are to be counted from the most mitotically active area.10 high power fields should be counted from the same area but need not to be contiguous. Poorly preserved area should be ignored.

Table 5:Scoring of mitotic count

Field diameter0.59mm	**Field diameter 0.44mm**	**score**
0-9	0-5	1
10-19	6-10	2
>20	>11	3

Table.6.Final grading of carcinoma breast

GRADE	**SUM OF POINTS**
I	3–5
II	6–7
III	8–9

5. Lymphovascular Invasion

Lymph node metastases is often accompanied by involvement of vascular spaces by tumor cells.So, in node negative cases , vascular invasion confers a poor prognosis for survival and a risk factor for local recurrence. Infiltration of tumor cells into the lymphovascular spaces in dermis is also associated with poor prognosis.

6. Necrosis

Tumor necrosis is defined as the “presence of confluent necrosis of any dimension in a section of invasive cancer that could be distinguished at intermediate magnification. It a significant predictor of time to recurrence and overall survival with 10-year follow-up[97]

7. Stromal features

Tumors that contain minimal stromal reaction tend to have the circumscription, poorly differentiated nuclear and histologic grade, and a prominent lymphoplasmacytic reaction. They also tend to be estrogen-receptor negative. On the other hand, densely fibrotic or scirrhous carcinomas are more likely to be stellate, moderately differentiated, and to have little lymphoplasmacytic reaction. A greater proportion of these lesions are estrogen-receptor positive.

8. Patient age

It is found that if age at the onset of disease is less than 50 years of age the patient has the best prognosis and the survival rate declines after the age of 50 years.

9. Family history

The presence of history of breast cancer in family is associated with poor prognosis.

10. Inflammatory cell infiltrates

Various studies have shown that prominent inflammation is associated with high histologic grade and better survival.When Grade 3 ductal NST carcinoma was associated with prominent inflammation it showed a better prognosis than those without prominent inflammation.[98] A subsequent study showed that tumor infiltrating CD8-positive T cells were associated with improved survival.[99]

11. Apoptosis

Apoptosis is a mode of cell death.It is characterized by cell shrinkage and rounding,chromatin condensation, fragmentation of the nuclei (pyknosis and karyorrhexis) with intracellular and extracellular chromatin fragments and cytoplasmic fragmentation with cytoplasmic blebs.High apoptotic index is shown to be associated with high grade, with a high proliferative activity, and with absence of hormone receptors.

12. Angiogenesis

Angiogenesis refers to proliferation of new capillaries from the existing vascular network. Angiogenesis is required for growth of the tumor and for metastases. Increased microvessel density in breast cancer has consistent association with lymph node metastasis and reduced survival in lymph node–negative breast cancer.[100,101]

13. Fibrotic foci

Fibrotic focus is a region seen in invasive carcinoma of breast that replaces the central necrotic area ,which is mostly made up of fibroblasts and collagen fibers.. This is found to be associated with basal subtype, an expansive growth pattern, hypoxia and angiogenesis, activated wound-healing signature and a poor prognosis.[102]

14. Perineural invasion

Perineural invasion is often seen in high-grade tumors which show vascular invasion and involvement of lymph nodes.[103]

MOLECULAR PROGNOSTIC AND PREDICTIVE FACTORS

A prognostic factor is defined as a characteristic of a patient or tumor at the time of diagnosis that can be used to provide information on clinical outcome (relapse and death) in the absence of therapy, whereas a predictive factor is defined as a characteristic that provides information on likelihood of response to therapy. In breast cancer, assessment of hormone receptors and HER-2 gives an idea about response of the malignancy to chemotherapy and endocrine therapy. The development of methods to detect antigens on tissue sections with antibodies was a major advance in surgical pathology[104,105]. Routine assessment of ER, PR, and HER-2 status on all primary invasive and recurrent breast cancers is recommended by the American Society of Clinical Oncology/College of American Pathologists.

Immunohistochemical (IHC) studies are most frequently used for classification of tumors,to identify insitu lesions/invasive lesions and to determine

prognostic and predictive factors.Estrogen and progesterone receptors are localized to the nuclei of epithelial cells.In normal resting breast the nuclei of approximately 7% of epithelial cells are immunoreactive for estrogen receptor while a higher proportion of cells show positivity in lobular than in ductal cells.[106,107]

Immunohistochemistry refers to process of employing antibodies as specific probes for the visualization of cell and tissue bound antigens. Immunoenzymatic techniques employing antibodies conjugated with enzymes are used to identify antigen-antibody reaction[108,109].Immunochemical approach for the demonstration of ER and PR is highly sensitive and specific.

ESTROGEN RECEPTOR

Estrogen receptor is a steroid receptor situated in the nucleus of the cell. Hormone diffuses in to the nucleus to bind to the receptor. The important function of the genes regulated by steroid receptors is monitoring cell growth and this effect of ER determines the behavior and treatment of breast cancer.

This receptor and can be identified by different methods like ligand binding, immunoassays, and immunocytochemistry. The inhibition of these receptors by various methods like oophorectomy, estrogen agonists (selective ER modulators) or indirectly by preventing the conversion of androgens to estrogen (e.g., aromatase inhibitors), forms a major target of breast cancer endocrine therapy.[110,111]

PROGESTERONE RECEPTOR

PR is an estrogen-regulated gene and it's positivity indicates a functioning ER pathway.Various data have shown that determination of PR assessment in carcinoma breast have definite prognostic and predictive significance[112]. PR-positive cancers are likely to have a better prognosis than PR-negative tumors and they can also predict response to endocrine therapy .[113]

Her-2/neu

Her2/neu is a proto-oncogene which encodes a transmembrane protein that shares homology with epidermal growth factor . Overexpression of Her2/neu in breast cancer implies that the tumor has worse prognosis than tumors which are Her2/neu negative[114]. The presence of Her2/neu is also used to predict whether patients respond to a monoclonal antibody to Her2/neu (Herceptin).[115]

IMMUNOHISTOCHEMISTRY

Immunohistochemistry (IHC) or immunocytochemistry is the application of immunologic principles and techniques to demonstrate specific antigens in cells and tissue based on the antigen antibody interaction and it exploit the specificity at light microscopic level.

Various stages of development of Immunohistochemistry include peroxidase–antiperoxidase method (1970), alkaline phosphatase labeling method(1971), avidin biotin method (1977) and two layer dextrin polymer technique(1993).

Steps of immunohistochemistry:

Antigen retrieval : Antigen retrieval is done to unmask the antigen determinants of fixed tissue sections.This can be done by

1. Proteolytic enzyme digestion

2. Microwave antigen retrieval

3. Microwave and trypsin antigen retrieval

4. Pressure cooker antigen retrieval

Proteolytic enzyme digestion:

Enzymes like trypsin and proteinases are used to breakdown the formalin cross linkages and unmask the antigen determinants. But there is a disadvantage of antigen destruction and inadequate digestion.

Microwave antigen retrieval:

In this formalin fixed paraffin sections are boiled in various buffers for rapid and uniform heating. Currently this is the most common method used.

Pressure cooker antigen retrieval:

In this method also the tissue sections are boiled in buffers to unmask the antigens. This method is used to retrieve large number of slides.

Detection systems: After adding specific antibodies to the antigens, the antigen antibody complex should be detected. This is done by direct and indirect methods.

Direct method:

The primary antibody is directly conjugated with flurochrome. Commonly used flurochromes are horse radish peroxidase and alkaline phosphatase.

Indirect method :

It is a two-step method .First the labeled secondary antibody reacts with primary antibody which is bound to specific antigen. The use of peroxidase enzyme complex or avidin biotin complex further increases the sensitivity of immunohistochemical stains.

IMMUNOCYTOCHEMISTRY

Immunocytochemical methods for ER and PR are readily adaptable to cytological materials including fine needle aspirates, touch imprints, malignant effusions, cytospins, and ThinPreps.[116] The ability to perform ER and PR in cytological preparations is particularly valuable for patient management issues, including patients who require preoperative chemotherapy and in cases of recurrent or metastatic breast cancer.

Interpretation of ER,PR and Her-2/neu

Many different methods are currently used to report the results of IHC studies for ER and PR. One method that is commonly used is the Allred score method[117]. Patients with carcinomas that scored 3 (<1% of cells with intermediate intensity or 1% to 10% of cells with weak intensity) or above had improved disease-free survival when treated with endocrine therapy.

Table.7. ER AND PR –ALLRED SCORE

Proportion score (PS)	% positive cells	Intensity score (IS)	Intensity of positivity
0	**0**	**0**	**None**
1	**<1%**	**1**	**Weak**
2	**1%-10%**	**2**	**Intermediate**
3	**10%-33%**	**3**	**Strong**
4	**33%-66%**		
5	**>66%**		

Total score(PS+IS)	Interpretation
0,2	NEGATIVE
3,4,5,6,7,8	POSITIVE

(Hammond ME, et al, American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Immunohistochemical Testing of Estrogen and Progesterone Receptors in Breast Cancer, J Clin Oncol 2010 Apr 19).

Table.8.HER2/neu score

SCORE	CRITERIA
0 (Negative)	No staining or if present,only in <10% of cells
1+ (Negative)	Faint or weak staining in >10% of tumor cells and only a portion of the membrane is positive.
2+ (Equivocal)	Weak to moderate staining in >10% of tumor cells and there is complete immunoreactivity.
3+(Positive)	circumferential intense and uniform membrane staining is shown by more than 30% of the tumor cells . A homogeneous (chicken wire) pattern should be present.

Recommended guidelines by ASCO

1. The type of fixative that can be used is 10% neutral buffered formalin
2. The ideal time of fixation should be between 6-48 hrs
3. The most reliable antibody is rabbit monoclonal antibody,4B5

If the results are 2+ it is recommended to perform FISH. Recently ASCO/CAP[118] has published their recommendations for HER2/neu testing.

The result is taken as positive in case of ,

1.3+ staining by IHC

2. More than 6 HER2/neu gene copies in FISH

3. FISH ratio >2.2

The result is taken as negative when there is

1. 0 or 1+ positivity by IHC
2. < 4 HER2/neu copies per nucleus by FISH
3. FISH ratio <1.8

PATHOLOGICAL AND CLINICAL IMPLICATIONS OF MOLECULAR SUBTYPING OF CARCINOMA BREAST

Breast cancer is known to be a heterogeneous disease .So tumors with the same clinicalpathological characteristics tend to exihibit diversity in disease behavior, response to therapy and prognosis. Gene expression profiling studies is supposed to be the gold standard method to subtype tumors based on molecular features.. As gene expression profiling from formalin-fixed, paraffin-embedded samples is not readily available at present ,IHC surrogate panels of estrogen receptor (ER), progesterone receptor (PR), HER2 and ki-67 can be readily used to subtype the malignant tumors of breast. (Nielsen et al., 2004; Livasy et al., 2006; Carey et al 2007; Hugh et al., 2007; Cheang et al., 2008).

The classification of breast cancer at the molecular level is according to hormone receptor expression and c-*erb*B-2 (HER-2) status. Other factors like lymphovascular invasion and expression of proliferation-associated markers (e.g., Ki67), p53, and E-cadherin may also have biologic and prognostic value.

With the use of modern technologies like microarray analysis, breast tumors can be divided in to five molecular subtypes- luminal A, luminal B, normal breast–like, HER-2, and basal[119].

A dysfunctional BRCA-1 protein is exihibited by a large number of basal tumors. These tumors express p53 or *TP53* gene mutation in majority of cases and also exihibit high proliferation indices identified by high number of mitoses or increased Ki67 labeling index.

Table.9.Molecular subtyping of carcinoma breast

	Luminal A	**Luminal B**	**HER2/*neu***	**Basal-like**
Gene expression pattern	Expression of luminal cytokeratins, and high expression of hormone receptors and associated genes	Expression of luminalcytokeratins, and moderate to weak expression of hormone receptors.	High expression of *HER2* and other genes in amplicon on 17q12 Low expression of ER and associated genes	High expression of basal epithelial genes, basal cytokeratins Low expression of ER and associated genes Low expression of HER2/*neu*
Clinical and biologic features	ER/PR positive & HER2/*neu* negative	ER/PR positive HER2/*neu*- variable Higher proliferation than luminal A tends to be higher histologic grade than luminal A	ER/PR negative HER2/*neu* positive High proliferation *TP53* mutation common More likely to be high grade and node positive	Most ER/PR and HER2/*neu* negative (‘triple negative’) High proliferation *TP53* mutation common; *BRCA1* dysfunction

Histologic correlation	Tubular carcinoma Cribriform carcinoma Low grade invasive ductal carcinoma NOS Classic lobular	Invasive ductal carcinoma NOS Micropapillary	High grade invasive ductal carcinoma NOS	High grade invasive ductal carcinoma NOS Metaplastic carcinoma Medullary carcinoma
Treatment response and outcome	Respond to endocrine therapy	Respond to endocrine therapy not as good as luminal A	Respond to trastuzumab (Herceptin)	No response to endocrine therapy or trastuzumab (Herceptin)
	Response to chemotherapy variable	Response to chemotherapy variable (greater than luminal A)	Respond to anthracycline-based chemotherapy	Appear to be sensitive to platinum-based chemotherapy and PARP inhibitors
	Good prognosis	Prognosis not as good as for luminal A	Generally poor prognosis	Generally poor prognosis (but not uniformly poor)

*(Modified from Schnitt SJ. Will molecular classification replace traditional breast pathology? Int J Surg Pathol 2010, **18:** 162S–166S; and Correa Geyer F, Reis-Filho JS. Microarray-based gene expression profiling as a clinical tool for breast cancer management: are we there yet? Int J Surg Pathol 2009, **17:** 285–302)*

PROGNOSTIC AND PREDICTIVE VALUE OF HORMONE RECEPTORS/Her-2/neu

High-dose systemic therapy which is given preoperatively in patients with locally advanced or inflammatory carcinoma is called neoadjuvant therapy.This can be either chemotherapy or endocrine therapy. Neoadjuvant chemotherapy takes an important place in treatment of operable breast cancer in the hope of improving conservative surgery rate of female patients. The major targets of NCT is to downstage the tumor load and to raise the number of surgeries which can conserve breast.

The role of ER,PR and Her-2/neu status in breast carcinoma has been extensively studied. Earlier it was believed that ER positive tumors had a better prognosis but now it has been established that although ER positive tumors are slow growing they do not have a lower metastastatic potential .The significance of ER positive tumors is that it predicts the response to tamoxifen. It is also noted that women who have an ER Allred score of 6, and above seem to respond better when given neoadjuvant hormone therapy.Also, aromatase inhibitors produce responses in tumors with lower levels of ER whereas tamoxifen does not. Many major clinical trials have shown that addition of Tamoxifen, a SERM, to conventional chemotherapeutic and surgical treatment protocols consistently improves disease free survival in women with hormone receptor-positive tumors.[120]

Her-2/neu over expression seems to be a weak to moderate independent predictor of survival.It acts as a reliable negative predictor for response to

alkylating agents and a moderately positive predictor of response to anthracyclines. In a hallmark study carried out by Slamon et al in 1987, HER2 amplification was verified as a significant independent negative predictor of overall survival and time to relapse [121]. Since then, HER2 status, as determined by either fluorescence in-situ hybridization (FISH) or IHC, has become important for prognostic implication and to assess potential response of patients to treatment with the monoclonal antibody trastuzumab (Herceptin). Several large, randomized clinical trials sponsored by the National Cancer Institute in 2005 have demonstrated that if chemotherapy and Herceptin are used together in patients with Her-2/neu positive breast cancer,it can drastically reduce the chance of recurrence as compared to those treated with chemotherapy alone.[122].

PROGNOSTIC IMPLICATIONS OF HR +/HER2-NEGATIVE BREAST CANCER

Under HR+/HER2-negative breast cancers, 90-95% of tumors belong to Luminal A and B subtypes. It is shown that in the early breast cancer, Luminal B disease has worse prognosis at 5- and 10-yrs inspite of adjuvant systemic therapy compared to Luminal A disease[123]. Apart from predicting baseline prognosis, the Luminal A vs B classification, along with tumour size and nodal status also estimates the residual risk of late recurrence at a distant site,ie at 5-10-years of follow-up.[124]Studies have also shown that the Luminal B tumours are more chemosensitive than Luminal A tumours[125] and Luminal A tumours which have a lower baseline proliferation status than Luminal B tumours seem to respond to endocrine therapy better.[126]

PROGNOSTIC IMPLICATIONS OF Her-2/neu POSITIVE CANCERS

The prognostic implications of *HER-2* gene amplification are (1)it predicts poor prognosis and response of tumors to systemic chemotherapy (2)HER-2 expression often increases with the absence of hormone receptors and vice versa. (3)

HER-2 positivity often seems to be associated with a relative resistance to endocrine therapies.(4)Over-expression of HER-2/neu implies that the tumor responds to trastuzumab (Herceptin)(5)It is also an important negative predictor of overall survival and time to relapse in patients with lymph-node-positive breast cancer (Suo et al., 2002). (6)It has also been shown that tumors with increased Her-2/neu expression is associated with a poor histologic grade and increased lymph node involvement. However, the prognostic implication of HER-2 positivity is more in node-positive cases as compared to node-negative cases..

Trastuzumab is a humanized monoclonal antibody against HER-2neu.It is known to have immense benefit in patients with HER-2–positive breast cancer.Thus with the advent of this drug, it is highly essential to determine the HER-2 status of breast cancer in pre-operative period to plan treatment. Trastuzumab is useful in searly-stage patients with Her2-positive breast cancer. The advantages of trastuzumab is that it reduces the risk of disease recurrence and improves overall survival.

PROGNOSTIC IMPLICATIONS OF TRIPLE NEGATIVE CANCERS

The features of Basal tumors (triple-negative lesions) are (1)poor disease-free survival and overall survival(2) distinct patterns of recurrence and distant metastasis(3) commonly seen in younger patients(4) they are generally high grade tumors with very high mitotic indices,abundant necrosis, tissue infarction, collagen, and hyaline material (5)have pushing borders(6) significant lymphocytic infiltrate; and typical or atypical medullary features.(7)their response to endocrine therapy or trastuzumab (Herceptin) is very poor.(8)They respond to PARP inhibitors(9)They usually show poor prognosis.

MATERIALS AND METHODS

The present study is a prospective study to assess and compare the role of Conventional FNAC and Liquid based cytology in the pre-operative diagnosis and prognosis of carcinoma of breast. . Approval of the Institute Ethical Committee was obtained to conduct this research study.

STUDY LOCATION

The study was conducted at the Department of Pathology, Tirunelveli medical college,Tirunelveli.

STUDY PERIOD

The study was conducted over a period from November 2014 to Septemder 2015 .

INCLUSION CRITERIA

FNAC smears diagnosed as carcinoma of breast.

EXCLUSION CRITERIA

1. FNAC smears diagnosed as Inflammatory lesions.
2. FNAC smears diagnosed as benign neoplasms.
3. FNAC smears diagnosed as atypical ductal hyperplasia.
4. FNAC smears of breast carcinoma patients on chemotherapy.

SAMPLE SIZE

A total of 30 cases of carcinoma breast diagnosed on FNAC were studied.

METHODOLOGY

The details of the patient on the request are verified. This is followed by the examination of the patient with special emphasis on features like location of the lesion, the presence or absence of skin or chest wall involvement, involvement of nipple, tumor size, consistency of tumor and circumscription of the tumor. The data are recorded in a format(Annexure 1)

PREPARATION OF SMEARS

CONVENTIONAL FNAC

A 22 to 27-gauge, 1 or 1.5-inch needle attached to a disposable 20-ml syringe is used to obtain breast aspirates. The material obtained through first pass is used to make four smears. One of them is stained with hematoxylin and eosin stain.

The remaining smears are used for immunocytochemical staining.

HAEMATOXYLIN & EOSIN STAINING TECHNIQUE

PREPARATION OF HAEMATOXYLIN SOLUTION:

Haematoxylin	2. 5gm
Mercuric oxide	1.25gm
Potassium alum	50gm
Absolute ethyl alcohol	l 25ml
Sodium iodate	0.5gm
Distilled water	500ml

PROCEDURE:

Potassium alum, 50gm is dissolved in 500ml of distilled water by heating

and shaking at 600 C. Add solution of 2.5gm of Haematoxylin in 25ml of absolute ethyl alcohol and bring rapidly to boil. When it begins to boil, remove from flame and add 1.25gm of mercuric oxide or sodium iodate. When the solution is cold add acetic acid.

PREPARATION OF EOSIN SOLUTION:

Eosin Y 1gm

95% Ethanol 80ml

Glacial Acetic acid 0.2ml

Distilled water 20ml

PROCEDURE:

Dissolve 1gm Eosin Y in 20ml of distilled water and add 80ml of 95% etanol and 0.2ml of glacial acetic acid.

STAINING PROCEDURE:

1.Xylene 3 changes-2mins each.

2.90%, 80%, 70% alcohol-10 dips each.

3. Bring sections to water.

4. Harris Haematoxylin-15 minutes.

5. Rinse in tap water.

6. Differentiate in 1% acid alcohol.

7. Rinse in tap water.

8. Lithium carbonate 0.5%- until blue.

9. Tap water wash.

10. Eosin-15secs to 2mins depending on age of Eosin.

11. Rinse in tap water.

12. Dehydrate in 95% alcohol.

13. Absolute alcohol-3 changes-10 dips each.

14. Xylene 2 changes-10 dips.

15. Mount in DPX mountant.

RESULTS:

Nuclei- Blue

Cytoplasm, RBCs, Keratin- Pink,

Eosinophil granules- Orange red

LIQUID BASED CYTOLOGY

The material obtained through second pass is rinsed in to U-prep cytopreservative solution. It is kept for a minimum of 30 minutes for fixation and then centrifuged. The supernatant is discarded and four smears are made from the sediment by centrifuging samples using NANOCYT .Let the slides dry before staining. One of them is stained with hematoxylin and eosin as described above.and the remaining three slides are used to do immunocytochemical analysis(ER,PR and Her-2/neu).

The slides are studied under light microscopy and the data are recorded. The conventional FNAC smears stained with Hematoxylin and Eosin are graded according to Robinson’s criteria.The cytomorphology of conventional FNAC smears and LBC are compared and analysed

IMMUNOCYTOCHEMICAL EVALUATION:

The three conventional smears and LBC smears for evaluation of ER PR and Her-2/neu are fixed in absolute alcohol for around 30 minutes. Rabbit Monoclonal antibody (Path insitu) is used to bind with primary antigen and is detected by adding secondary antibody conjugated with horse radish peroxidase – polymer and diaminobenzidine substrate.

PROCESSING FOR IMMUNOCYTOCHEMISTRY

- The smears for ICC in conventional FNAC are made on ordinary glass slide while those in LBC are made on Poly Lysine coated adhesive slides.
- The three conventional smears and LBC smears for evaluation of ER PR and Her-2/neu are fixed in absolute alcohol for a minimum of 30 minutes.
- The slides are then kept in TRIS wash buffer for around 2 minutes.
- Endogenous peroxidase activity is removed by incubating the tissue sections with enough drops of 3% peroxide block in a humidity chamber for 5minutes.The sections are then washed in TRIS wash buffer.
- The smears are incubated with primary antibody for around 30 minutes.
- The tissue sections are then washed in 2 changes of TRIS wash buffer.
- The smears are treated with target binder for 15 minutes.
- The tissue sections are then washed in 2 changes of TRIS wash buffer
- The smears are treated with HRP-polymer DAB for 15 minutes.
- The tissue sections are then washed in 2 changes of TRIS wash buffer

- ❖ DAB chromogen (1ml DAB buffer +1 drop DAB chromogen) is then added over the smears and incubated for 4 minutes and then washed with 2 changes of distilled water.
- ❖ Counterstaining was done with haematoxylin for 30 seconds and washed in running tap water.
- ❖ Dehydration is done by 2 changes of 100 % alcohol.
- ❖ Mounting is done by DPX mountant and observed under microscope

BUFFER PREPARATION

Tris wash buffer

Tris - 0.605 gm

Sodium chloride - 8 gm

1 N Hcl - 4ml

Distilled water - 1000 ml

PRECAUTIONS

1. The glassware used should be dry and clean.
2. The buffer used should be prepared fresh and the p H should be adjusted according to the preferred p H.
3. The staining procedures are never allowed to dry so they are performed under a humidity chamber.
4. DAB chromogen should be handled and disposed carefully as it is a carcinogen.
5. Primary antibody, DAB chromogen, peroxidase block etc should be stored at 4-6ºC.

6. Then the slides are counterstained with Mayers Hematoxylin.

Scoring of ER and PR are done using Allred score (Table.7) and Her-2/neu scoring is done as per ASCO guidelines. Membrane staining with 3 + and 2+ immunoreactivity were considered HER2/neu positive. (Table.8)

Statistical Analysis

The SPSS software version 11 (SPSS, Chicago, IL, USA) was used to perform the statistical analysis of the data.. Mcnemar test ,which is a statistical test used on paired nominal data has been used to evaluate the statistical difference between the two methods. p value of <0.05 is considered significant.

OBSERVATION AND RESULTS

The study analysed the conventional fine needle aspiration cytology samples of 30 cases of malignant lesions of breast along with the corresponding liquid based cytology samples to compare the cytomorphology. The study also included the assessment of hormone receptor and Her-2/neu status on conventional FNAC smears and liquid based cytology and assess the diagnostic and prognostic value of conventional and liquid based cytology.

Table.10.Distribution of Grading of breast cancer in conventional smears

Carcinoma breast	Total No. of cases(n=30)
Grade-1	10
Grade-2	13
Grade -3	7

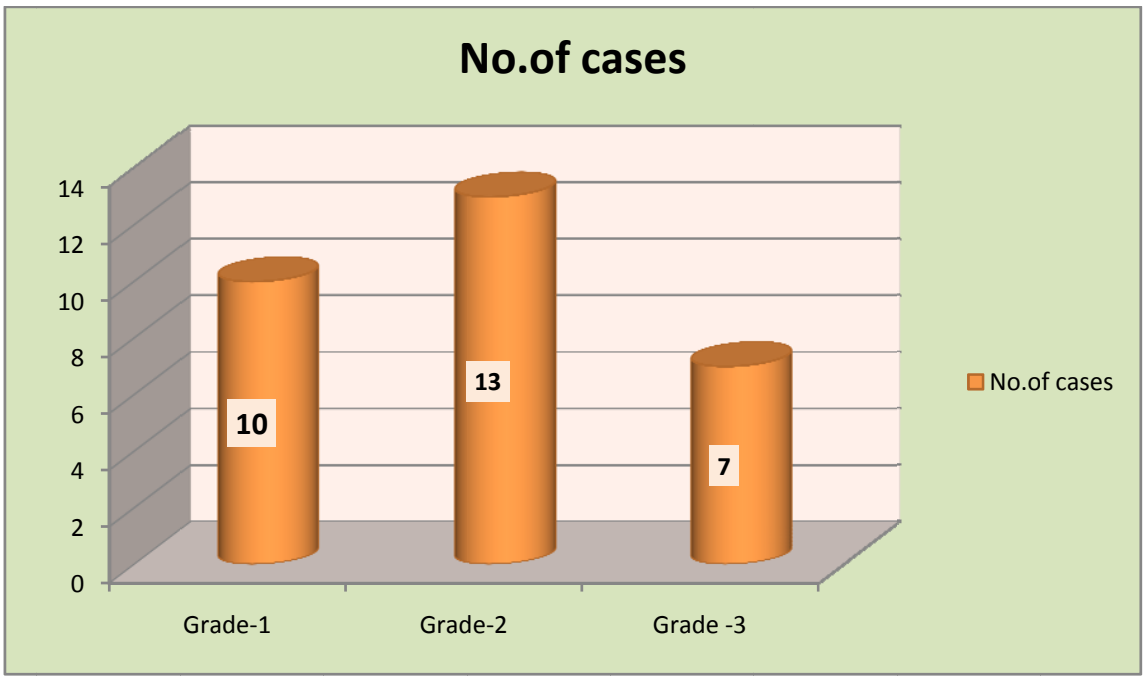

Chart.1. Distribution of grade of breast cancer in FNAC

In the present study,the number of cases which fall under Grade-1 are 10,under grade-2 are 13 and those under grade-3 are 7.

1. Comparison of cytomorphology on FNAC and LBC

The cytomorphology of FNAC samples and LBC samples are compared based on the following parameters-cellularity, background blood-debris, monolayers, cell architecture, nuclear details and cytoplasmic details.

1. CELLULARITY

Table.11.Comparison of cellularity in conventional FNAC

CELLULARITY (CONV.FNAC)	GRADE-1	GRADE-2	GRADE-3	TOTAL
POOR	3	2	2	7
ADEQUATE	4	11	6	21
GOOD	1	1	-	2

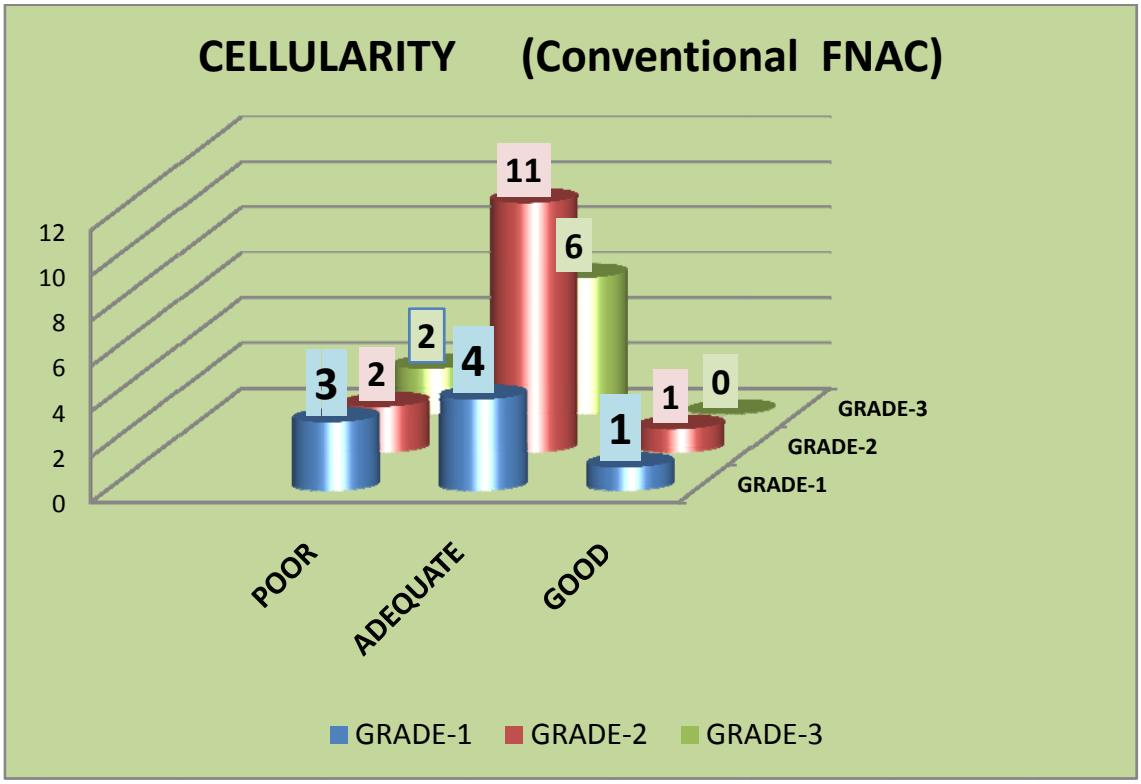

Chart.2.Cellularity in conventional FNAC

The cellularity in smears of conventional FNAC is compared among various grades of carcinoma breast. The grade of the tumor does not seem to have influence on the cellularity. In conventional FNAC, it was found that 7 cases had poor cellularity, 21 cases had adequate cellularity and 2 cases had good cellularity.

Table.12. Comparison of cellularity in LBC

CELLULARITY (LBC)	GRADE-1	GRADE-2	GRADE-3	TOTAL
POOR	1	1	2	4
ADEQUATE	6	8	6	20
GOOD	1	5	-	6

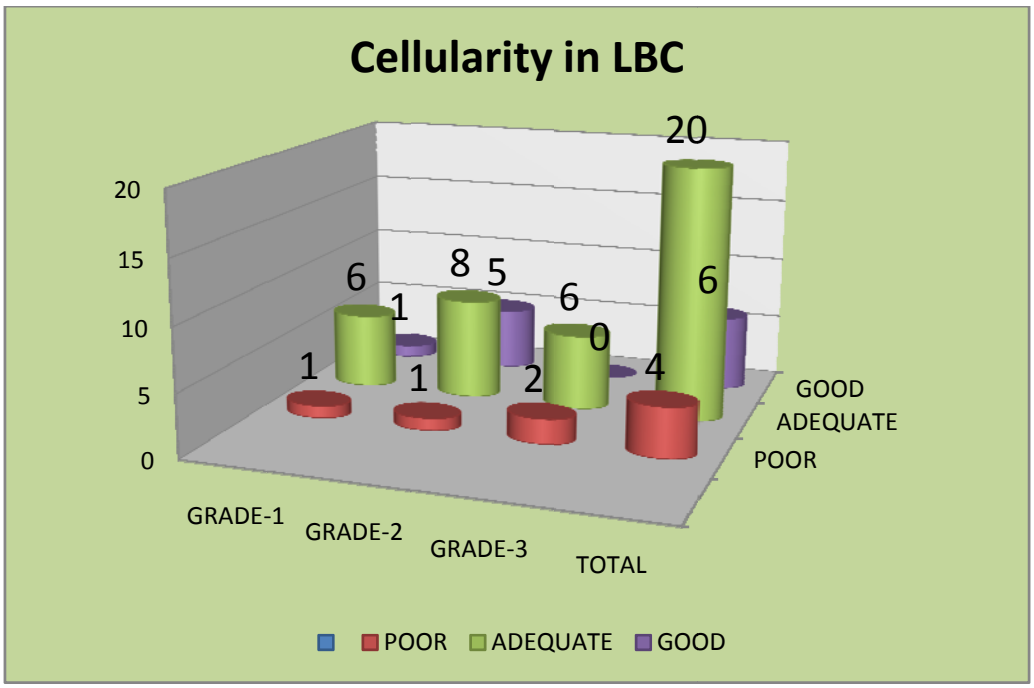

Chart.3.Cellularity in LBC

In LBC, the number of cases with poor cellularity is around 4,the number of cases with adequate cellularity is 20 and the number of cases with good cellularity is 6.In LBC also it is found that the grade of the tumor does not influence the cellularity of tumor.

Table.13.Percentage of cases with poor cellularity in FNAC and LBC

POOR CELLULARITY	No.of cases	%
FNAC	7	23.3 %
LBC	4	13.3%
COMBINED	1	3.3%

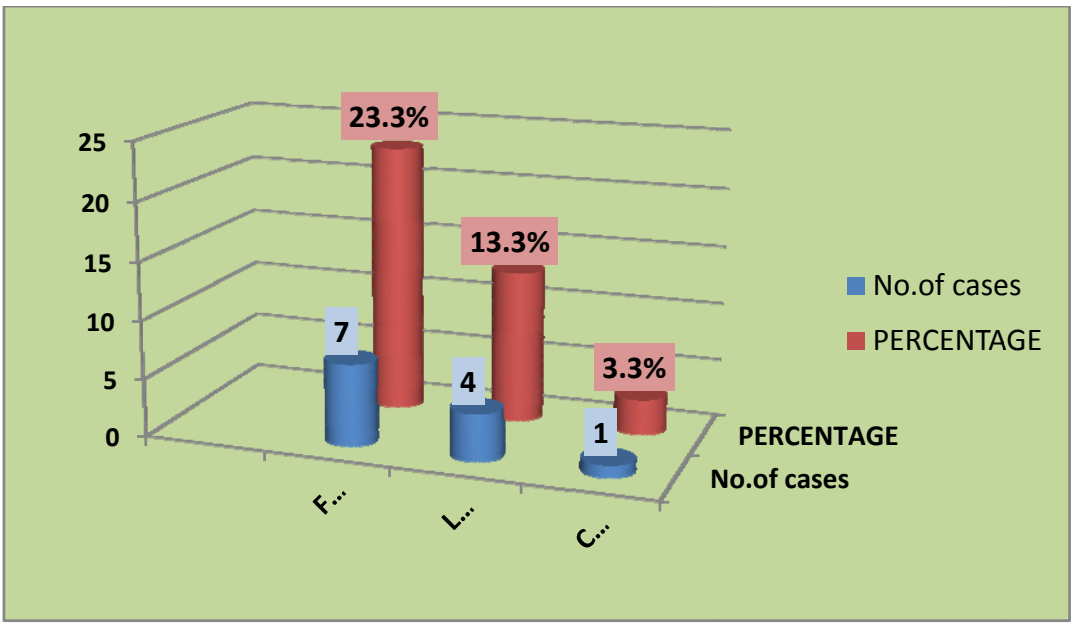

Chart.4..Percentage of cases with poor cellularity in FNAC and LBC

The percentage of cases with poor cellularity in conventional FNAC is 23.3%,while that in LBC is 13.3%.When the two methods were combined the number of cases with poor cellularity dropped down to 3.3%.

2. BACKGROUND BLOOD-DEBRIS

Table.14.Amount of background blood-debris in smears

BACKGROUND BLOOD-DEBRIS	zero	occasional	abundant
Conventional	4	10	16
LBC	12	15	3

The number of cases with abundant background blood-debris in conventional smears is about 16 while only 3 cases in LBC had abundant background blood-debris. The number of cases with no background blood-debris in LBC is around 12 while 4 cases in conventional smears had no background blood-debris.

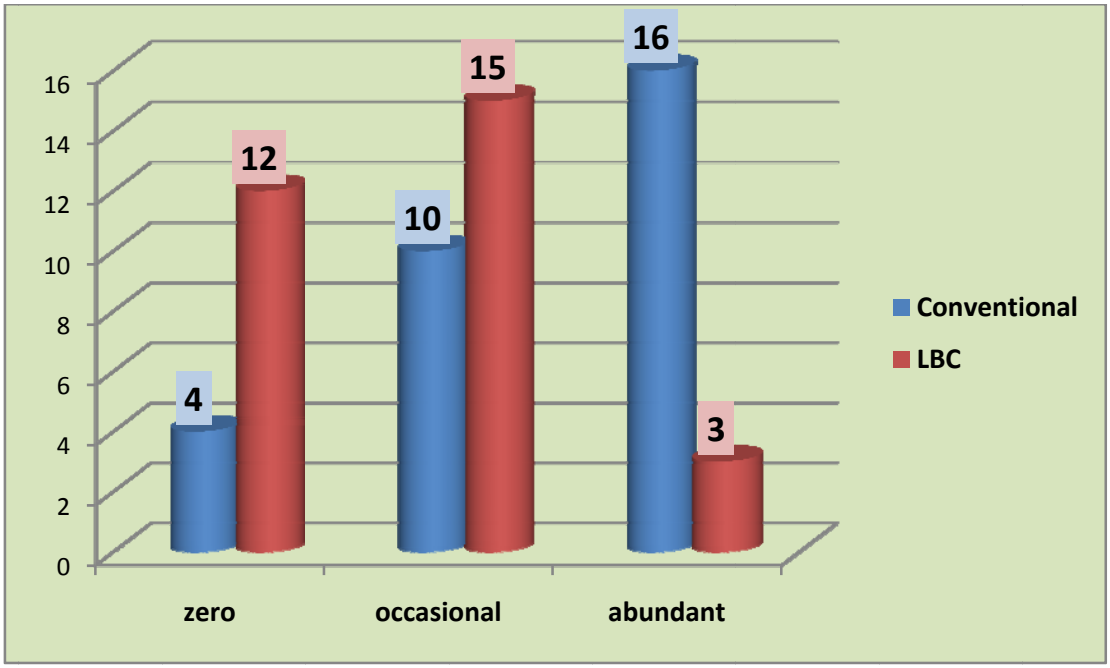

Chart.5.Amount of background blood debris in conventional FNAC and LBC

Table.15.Percentage of cases with abundant background blood debris in LBC and FNAC

Abundant background blood debris	No.of cases	%
CONV.FNAC	16	53.3%
LBC	3	10%

The number of cases with abundant background blood-debris in conventional smears is about 16 which constitutes around 53.3%while that in LBC is around 3 which constitutes only 10%.

3. MONOLAYERS

Table.16.Monolayering in conventional FNAC and LBC

Monolayer	Absent	Occasional	Good amount
Conventional	8	12	10(33.3%)
LBC	4	11	15(50%)

This study shows that 33.3% of cases had good amount of monolayering in conventional smears while 50% of cases had the same in LBC.

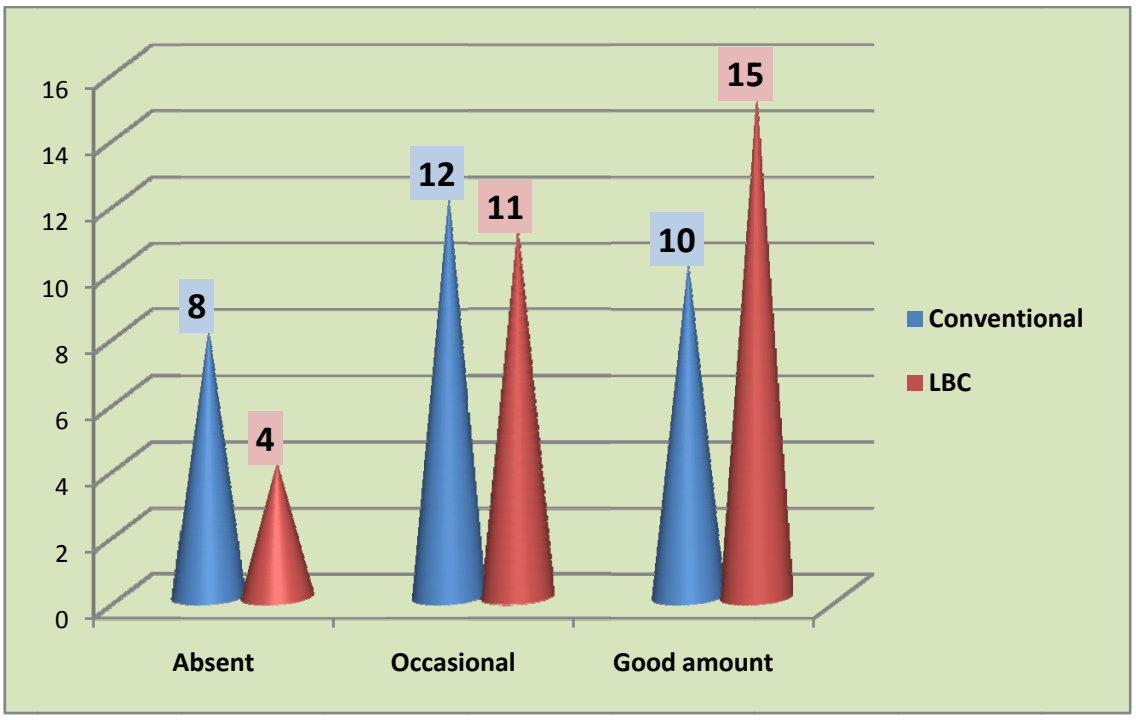

Chart.6. Monolayering in Conventional FNAC and LBC

4. CELL ARCHITECTURE

Table.17.Cell architecture in conventional FNAC and LBC

CELL ARCHITECTURE	Non- recognised	Moderately recognised	Well-recognised
Conventional	4(13.3%)	13	13
LBC	7(23.3%)	15	8

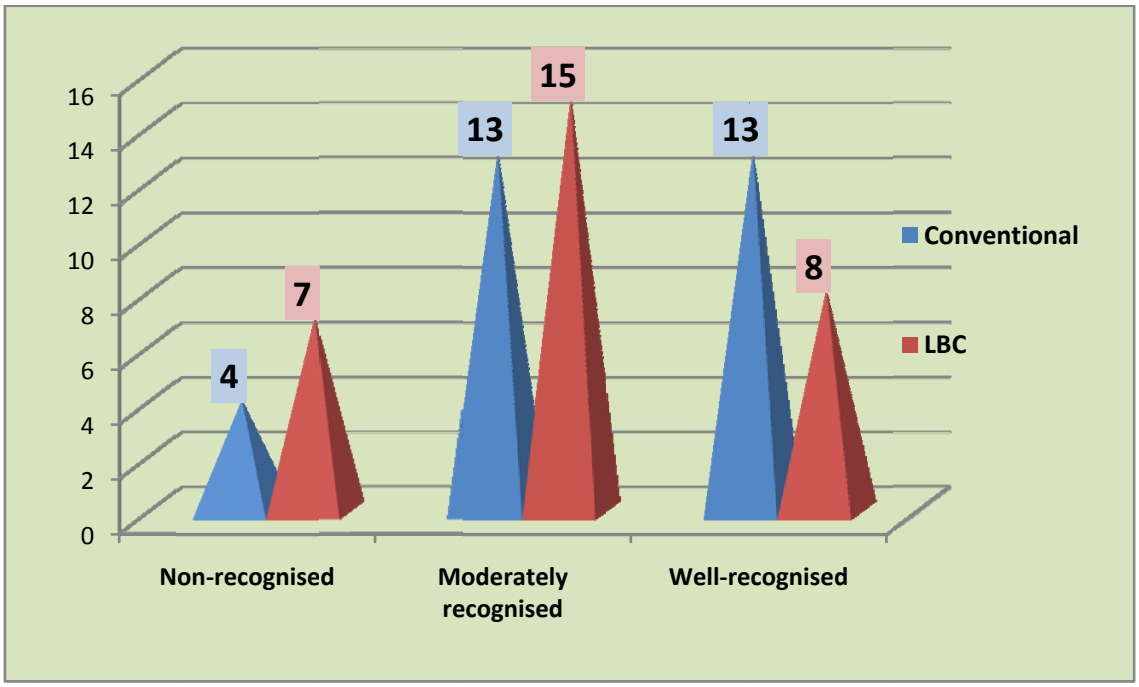

Chart.7. Cell architecture in conventional FNAC and LBC

The number of cases with non-recognised architecture in conventional FNAC are 4(13.3%) and the number of cases with moderately recognized and well recognized architecture is around 13 each. The number of cases with non-recognised architecture in LBC are 7(23.3%),while the number of cases with

moderately recognized and well recognized architecture is around 15 and 8 respectively.

6.CYTOPLASMIC AND NUCLEAR DETAILS

Table.18. Cytoplasmic and nuclear details in FNAC and LBC

CYTOPLASMIC AND NUCLEAR DETAILS	POOR	GOOD	EXCELLENT
CONVENTIONAL FNAC	3	20	7
LBC	5	18	7

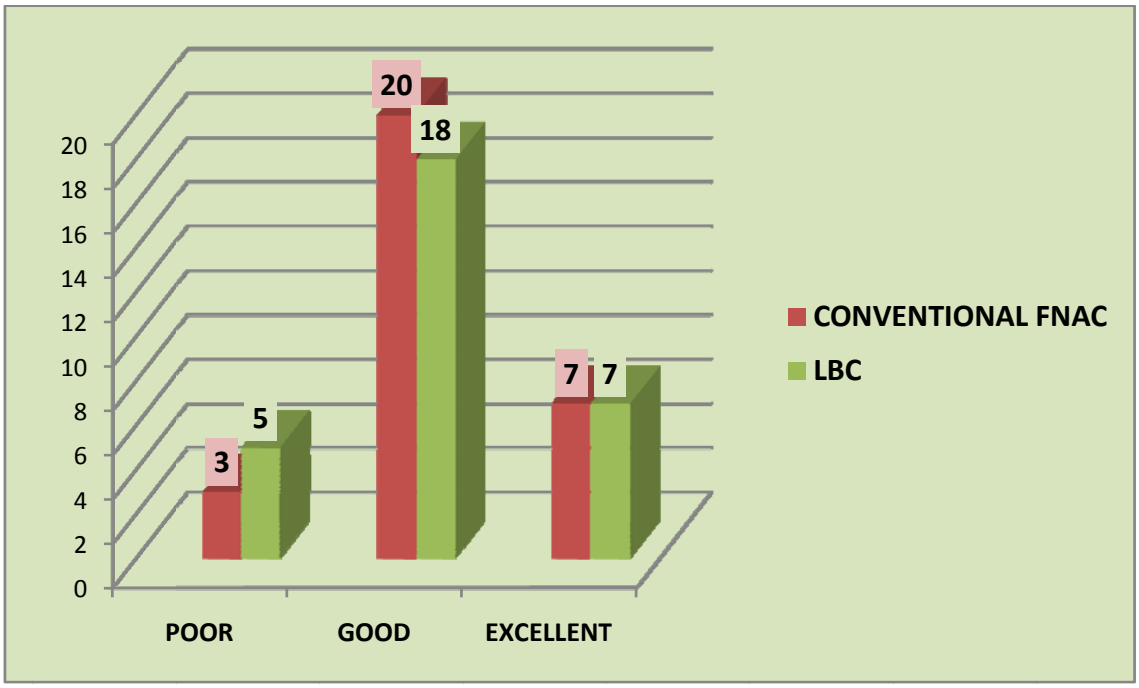

Chart.8. Cytoplasmic and nuclear details in FNAC and LBC

In the present study,the number of cases which had poor cytoplasmic and nuclear details in conventional FNAC were 3,while the number of cases which had poor cytoplasmic and nuclear details in LBC were 5.

8.Immunocytochemistry on Conventional FNAC .

Immunocytochemistry was performed on conventional smears of all 30 cases.The ER,PR and Her-2/neu status of the cases were analysed.Molecular subtyping was done based on this.The number of tumors with ER/PR positive,Her-2/neu negative are 9,those with ER/PR positive or negative and Her-2/neu positive are 10 and there are 11 triple negative tumors.

Table.19.ER/PR/Her-2/neu status of Carcinoma breast in different grades of tumor

TUMOR GRADE	**HR+ Her-2/neu-**	**HR+/- Her-2/neu+**	**TRIPLE NEGATIVE**
GRADE I	8	2	-
GRADE II	1	7	5
GRADE III	-	1	6

9. .Immunocytochemistry on LBC

Immunocytochemistry was done on LBC smears .It was noticed that the intensity of staining was very poor in immunocytochemistry done on LBC. The cases which were strongly positive in conventional smears were found to be negative in LBC smears. So LBC was not found to be as effective as conventional FNAC for immunocytochemistry.

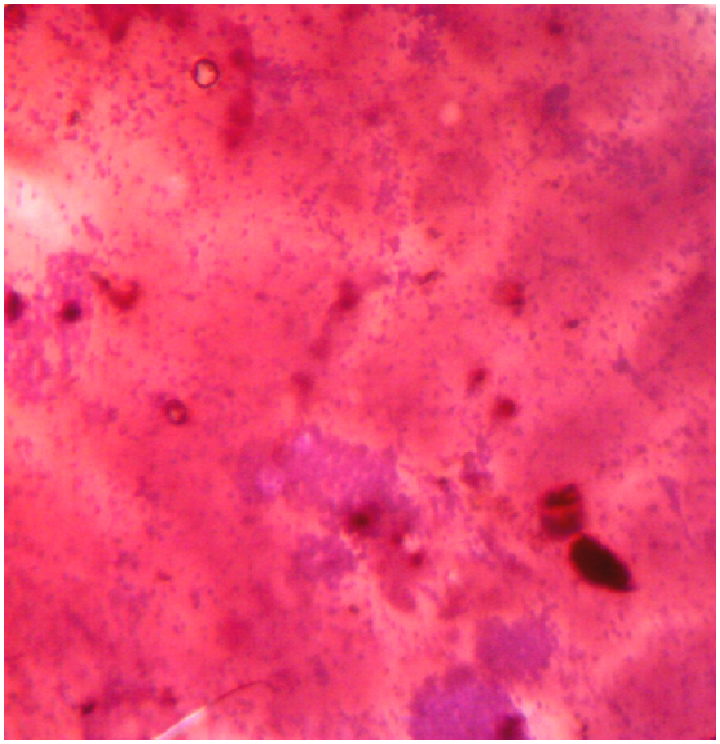

Fig.5.Conventional FNAC-100X-HEMATOXYLIN AND EOSIN

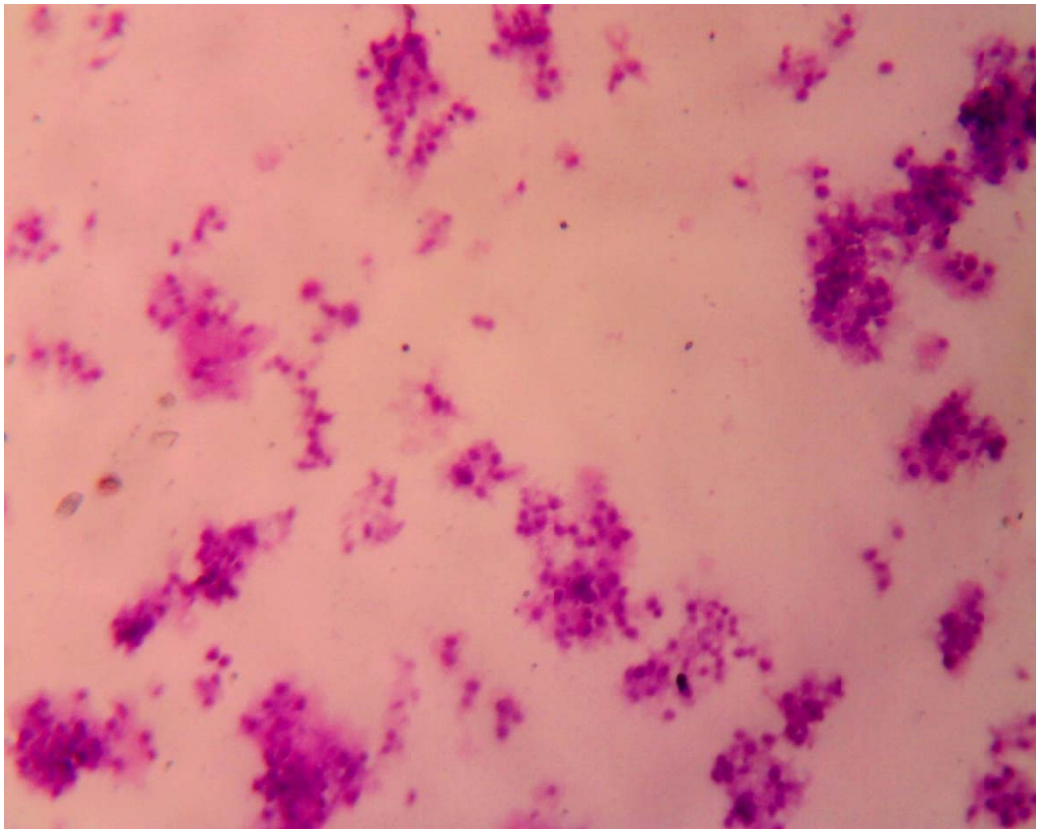

Fig.6 LBC.100X. HEMATOXYLIN AND EOSIN

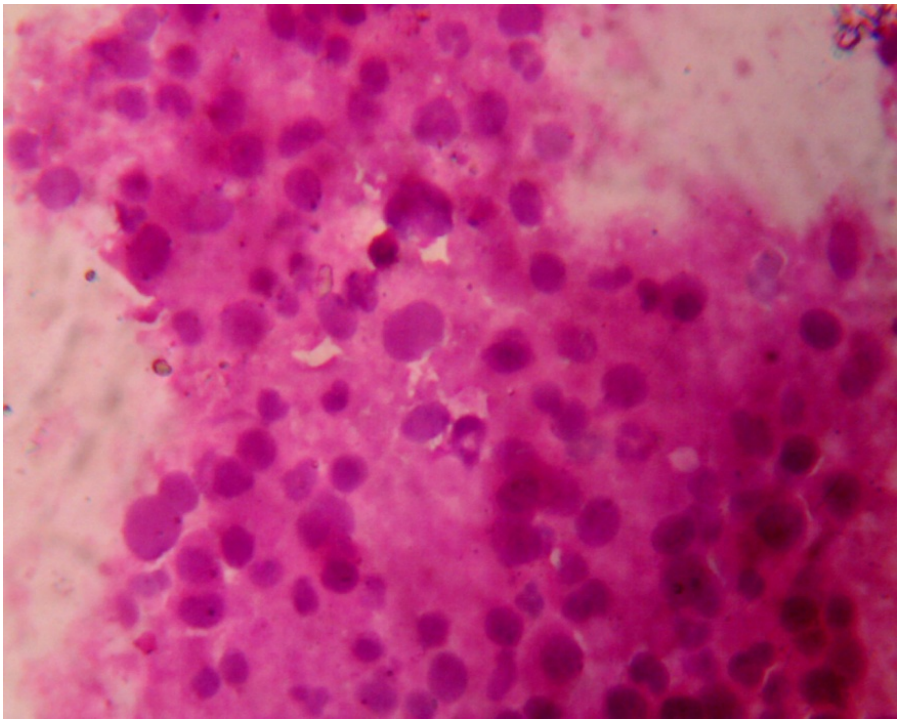

Fig.7.Liquid based cytology-400X - HEMATOXYLIN AND EOSIN

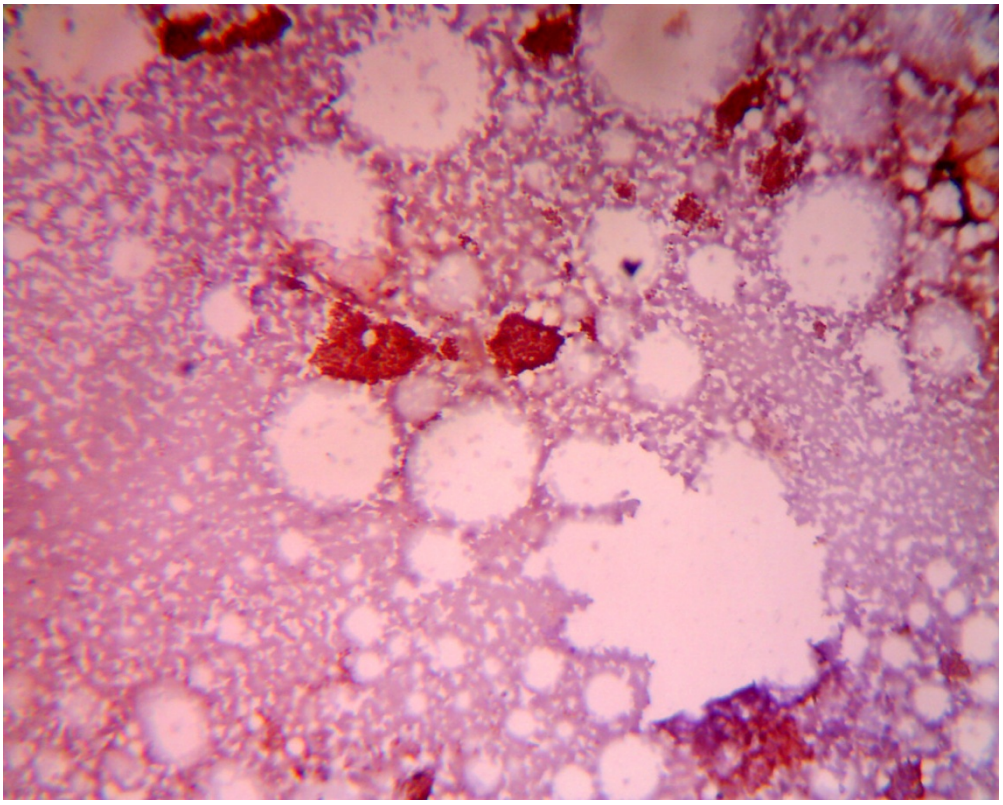

Fig.8. ESTROGEN RECEPTOR CONVENTIONAL FNAC -40X

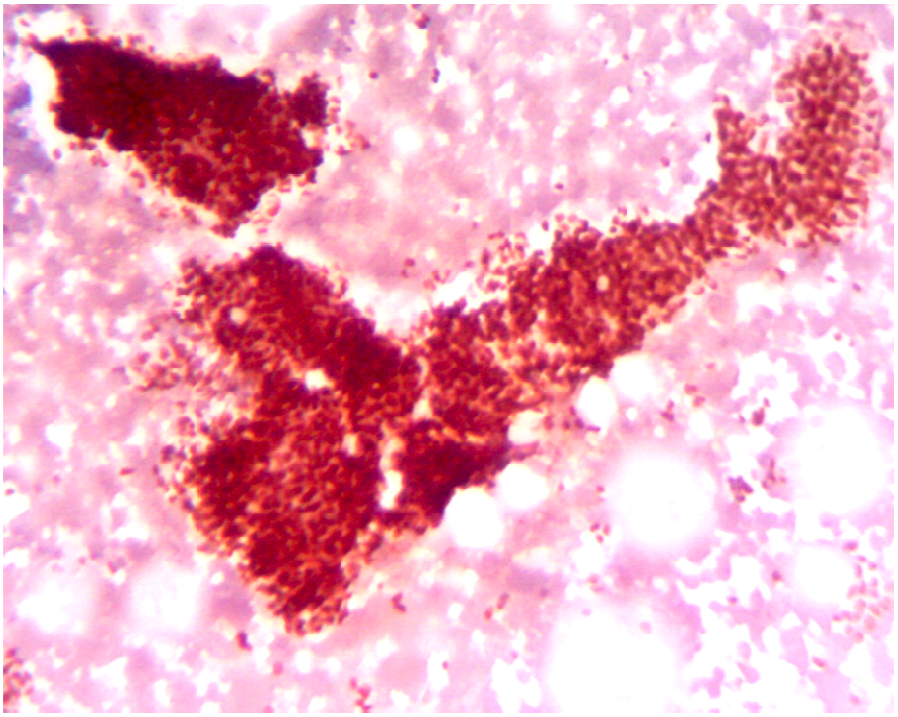

Fig..9 . ESTROGEN RECEPTOR CONVENTIONAL FNAC -100X

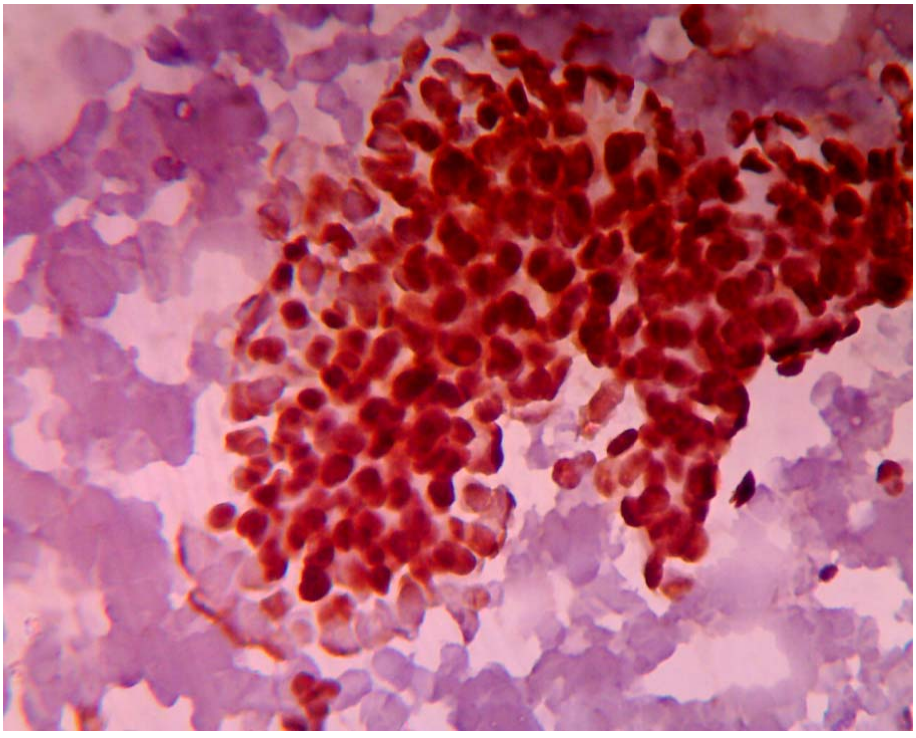

Fig.10 . ESTROGEN RECEPTOR CONVENTIONAL FNAC -400X

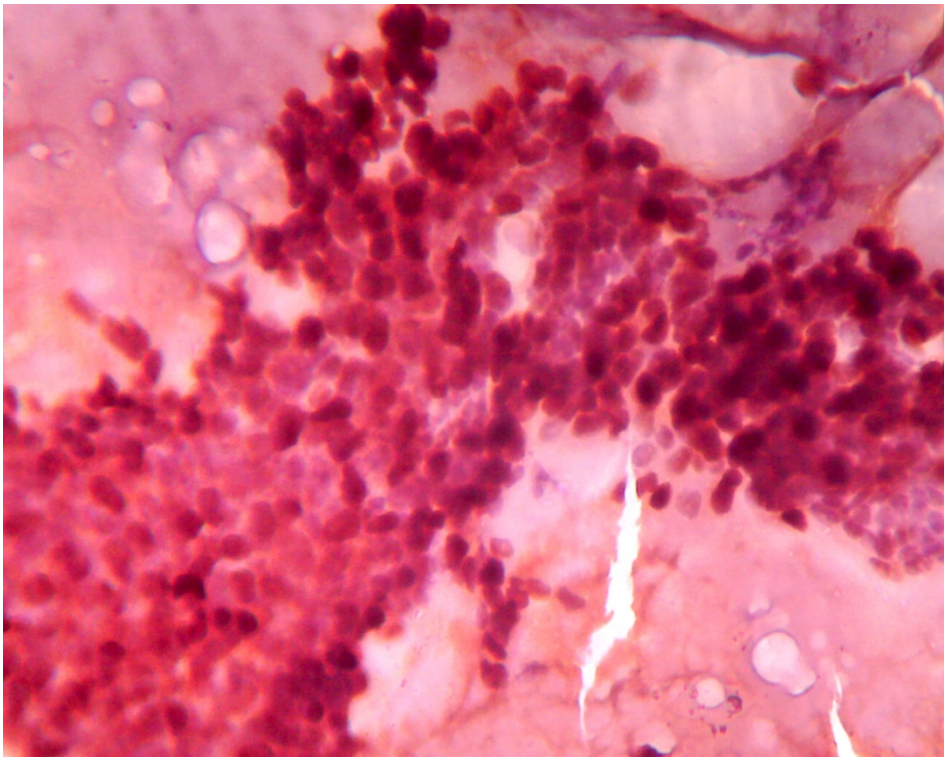

Fig.11.PROGESTERONE RECEPTOR CONVENTIONAL FNAC-400X

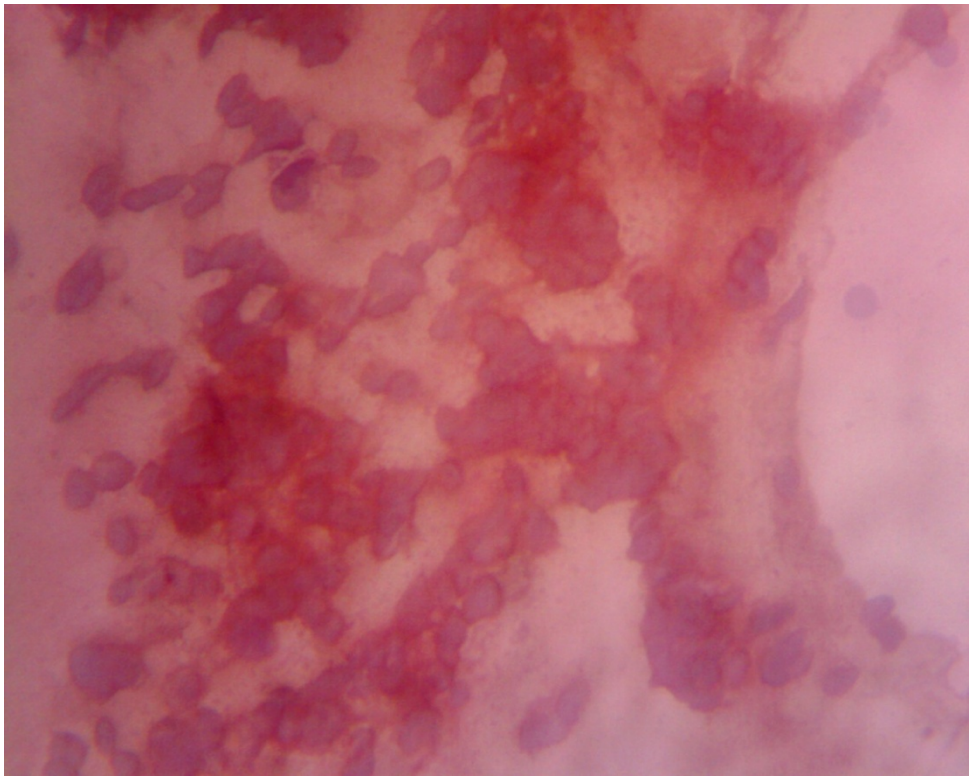

Fig.12.Her-2/neu CONVENTIONAL FNAC- 400X

DISCUSSION

Breast cancer is now considered as a heterogeneous disease which can be divided into distinct molecular subtypes that has prognostic significance. FNAC is a rapid, cheap, atraumatic procedure that does not require admission of the patient in the hospital. This method is found to have good accuracy and sensitivity, and is also a relatively simple procedure to procure tumor cells for ancillary studies, such as prognostic and predictive biomarkers.

In the present study, the cytomorphology of breast malignancy in conventional FNAC and liquid based preparations stained by H&E Stain are compared and analysed.Immunocytochemistry was performed on conventional FNAC smears and molecular subtyping of carcinoma breast was done.Immunocytochemistry on LBC was not very useful.In our study,30 cases of FNAC proven malignant lesions of breast were studied.

Table.20.Number of cases studied in various studies

Study	year	No.of cases
H Sartelet.et.al	2002-2003	103
Kyoko komatsu.et.al	2006-2007	9
Minhao.Lv.et.al	2006-2011	102
Present study	2014-2015	30

1. Grading of carcinoma breast in FNAC

In Fine needle aspiration cytology, carcinoma breast can be divided in to Grades 1,2 and 3 based on cell size, arrangement, pleomorphism, nuclear membrane, nuclear chromatin and nucleoli. In the present study, there were 10 cases of Grade 1 tumors,13 cases of grade 2 tumors and 7 cases of grade 3 tumors. According to a study by Ahmed Wani.et.al on 110 cases of breast malignancy which was graded on FNAC by Robinson's method,25.45% cases were grade I tumors,41.81 % cases were grade II and 32.72% were grade III .In the present study,33.3 % cases were grade I,43.3% cases were grade II and 23.3% cases were grade III.

Table.21.Comparison of grading of carcinoma breast with other study

Study	Grade1	Grade2	Grade3
Ahmed Wani.et.al	25.45%	41.81%	32.72%
Present study	33.3%	43.3%	23.3%

2.Cytomorphology of breast malignancy in Conventional FNAC and Liquid-Based Cytology

The cytomorphology of breast malignancy in conventional smears and liquid based cytology was compared using a semiquantitative system which analysed cellularity, background blood and cell debris, presence of cells in monolayers, cell architecture , and nuclear/cytoplasmic details.

A.CELLULARITY

Table.22.Comparison of adequate cellularity in FNAC and LBC

Study	Adequate/good cellularity (conventional FNAC)	Adequate/good cellularity (LBC)
Ryu.et.al	68.3%	76.7%
Present study	76.7%	86.7%

The percentage of cases which had adequate/good cellularity in conventional FNAC was around 76.6 % while that in LBC was 86.6% .The number of cases which had poor cellularity in conventional FNAC is 7 while the number of cases with poor cellularity in LBC is around 4. In the present study, the p value found using Mcnemar test is around 0.508. Thus, the cellularity in LBC was found to be superior as compared to that in conventional smears. A study by Ryu.et.al showed that the number of cases with adequate to good cellularity in conventional FNAC was around 68.3%,while that in LBC was 76.7%.The present study shows better yield of cellularity in conventional as well as Liquid based cytology as compared to the study by Ryu.et.al. A study by Michael. C.W et.al [130] also showed that the cellularity in LBC was found to be slightly superior as compared to that in conventional smears. Another study by Daskalopoulou.et.al also showed that there is great increase in cellularity in LBC aspirates of breast. Also,the percentage of cases with poor cellularity in conventional smears was found to be 23.3%,in LBC it was 13.3% and when both the methods were

combined,it reduced to 3.3%.Thus,if both the methods are used together,the cellular yield is much higher.

B.BACKGROUND BLOOD DEBRIS

In the present study, the percentage of cases with abundant background blood debris in conventional FNAC was 53.3% and that in LBC was 10%.The p value found by Mcnemar test is 1.Thus it is inferred that LBC is better than conventional FNAC with less amount of back ground material. In a similar study by Ryu.et.al the percentage of cases with background debris in conventional FNAC was 68.3%,while that in LBC was 26.7%. Other studies by Michael.C.W et.al [130] and Leung.C.S et.al[131] also showed that background is clear in LBC. However, there is loss of extracellular material such as mucus and stromal fragments in LBC which may be disadvantageous because it results in loss of informative background.Thus,LBC is superior to conventional smears with respect to clean background.

C.MONOLAYERS

The present study shows that the percentage of cases with good monolayering in conventional FNAC is 33.3% while that in LBC is 50%.The Mcnemar test shows that the p value is around 0.307.Thus the study shows that LBC smears has more monolayering than conventional smears. A study by Gerhard.et.al also showed that the LBC smears had less overlapping and more monolayering than conventional smears.

D.CELL ARCHITECTURE

In the present study, the number of cases with non-recognised architecture in conventional FNAC are 4(13.3%), while the number of cases with non-recognised architecture in LBC are 7(23.3%). The p value by Mc nemar test was found to be 0.210. This is analogous to the study conducted by Michael CW.et.al and Feoli.F.et.al which also showed that cellular aggregates are fragmented, shortened and less distinct in LBC preparations, resulting in smaller clusters and cellular dissociation which leads to poor preservation of cell architecture in LBC as compared to that in conventional FNAC.

E. CYTOPLASMIC AND NUCLEAR DETAILS

In the present study 10% of the smears in conventional FNAC showed poor cytoplasmic and nuclear details while 16.6% of smears in LBC showed poor cytoplasmic and nuclear details. The p value by Mcnemar test was found to be 0.727. This is analogous to the study done by Simsir.A.et.al which showed that cells in LBC are more rounded and smaller than the flattened cells of conventional smear because the fixative used in LBC is a liquid medium. According to Reyes N.et.al cells in LBC are poorly preserved and nuclei are swollen. However, in a study done by Dey P, it was shown that in LBC the cells are better-preserved with enhanced nuclear detail. A study by Biscotti.C.V.et.al also showed that there is better-preservation of cells with enhanced nuclear detail, including a well-defined, usually more pronounced, nucleoli in LBC.[132] Myoepithelial cells are inconspicuous in LBC smears.

Thus, the present study shows that LBC is superior to conventional FNAC in terms of cellularity, clean background and monolayers while the cytoplasmic and nuclear details in LBC are not as well preserved as in conventional smears.The present study points out that although the conventional FNAC and LBC have their own advantages and shortcomings, they have comparable diagnostic accuracy and a combination of these methods can be practiced to achieve a higher diagnostic rate.

This was analogous to study conducted by Dey P *et al.* which suggested that LBP and CS have comparable diagnostic accuracy in the evaluation of breast FNABs. Another study by Bédard YC *et al.* *which* analyzed 7464 breast FNABs over a 4-year period, comparing CS with LBP also found that there was no significant difference in diagnostic accuracy.

IMMUNOCYTOCHEMISTRY IN FNAC AND LBC

In the present study it was found that the number of tumors with ER/PR positive,Her-2/neu negative are 9,those with ER/PR positive or negative and Her-2/neu positive are 10 and there are 11 triple negative tumors. Of the 10 Grade I tumors, 8 cases are HR+/Her-2/neu negative and 2 cases are HR+/- and Her-2/neu positive. Of the 13 Grade II tumors,1 tumor was HR+/Her-2/neu negative,7 tumors were HR+/-,Her-2/neu positive and 5 tumors were triple negative. In Grade III tumors,1 tumor was HR+/- and Her-2/neu positive and 6 tumors were triple negative. Thus the study shows that majority of Grade I tumors are ER/PR+ and Her-2/neu negative, while majority of Grade II tumors were either Her-2/neu positive or triple negative and most of the Grade III tumors were triple

negative.Thus the prognosis of tumors based on grading correlates with the prognosis determined by expression of hormone receptors and Her-2/neu.

A study by Maynard et al., 1978 and Hilf et al., 1980 also showed that tumors which are ER and PR positive tend to be better differentiated and are likely to have better prognosis..A study by Slamon et al.,1987 and Tsuda et al.,1991 also showed that Her-2/neu positive tumors are commonly found to have a poor histologic grade.

Neoadjuvant chemotherapy or preoperative systemic therapy is useful in patients with operable breast cancer. Neoadjuvant chemotherapy increases the scope for breast conserving surgery .It also helps to achieve a pathological complete response (pCR; i.e. absence of malignant cells at tumor site.) and thereby increased disease free period and overall survival.

The evaluation of hormone receptors and Her-2/neu status in FNAC smears has largely helped to predict the response to neoadjuvant therapy and thereby plan the management protocol for the individual patient.

However, immunocytochemistry on LBC smears were found to be negative in all the cases which were strongly positive in conventional smears. The intensity of staining was very poor. The cause for the failure of the procedure is to be investigated and further standardisation of the procedure is essential. Thus, in the present study immunocytochemistry on LBC is found to be not as effective as in conventional smears.

SUMMARY

- This study includes a total of 30 FNAC proven cases of carcinoma breast.
- The cellularity in Liquid based cytology is found to be higher than that in conventional FNAC.
- Combined use of Liquid based cytology and conventional FNAC has better diagnostic value in carcinoma breast.
- The background blood debris is lower in Liquid based cytology than that in conventional smears.
- Monolayering of cells is more common in Liquid based cytology.
- The cytoplasmic and nuclear details is better preserved in conventional FNAC than in Liquid based cytology.
- In LBC,there are smaller clusters of cells and cellular dissociation is present.
- .Immunocytochemistry can be done on conventional FNAC smears and thereby subtyping of breast cancer is possible pre-operatively thereby judicious planning of neo-adjuvant chemotherapy/hormone therapy as well as assessment of prognosis of the tumor is possible during the pre-operative period itself.

CONCLUSION

In our study, it is concluded that although FNAC and LBC has it's own merits and demerits , they have comparable diagnostic accuracy and the use of combination of these methods has superior value in diagnosing breast malignancy than either of them alone. Immunocytochemistry on conventional FNAC can serve as a reliable tool in the sub typing of breast malignancy. This helps in efficient planning of neo-adjuvant chemotherapy during the pre-operative period and has definite prognostic value. However, in this study Liquid based cytology was found to be not as effective as conventional FNAC in determining the prognostic/predictive factors of carcinoma breast.

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ANNEXURE-1

CASE PROFORMA

Case number :

Name :

Age :

Sex :

HPE number :

IP number :

Clinical history :

Family history

Risk factors, if any :

Clinical diagnosis :

Imaging :

Previous FNAC

ANNEXURE-II

WHO HISTOLOGICAL CLASSIFICATION OF TUMORS OF BREAST

Epithelial tumors

Invasive ductal carcinoma,not otherwise specified

- Mixed type carcinoma
- Pleomorphic carcinoma
- Carcinoma with osteoclast like giant cells
- Carcinoma with choriocarcinomatous features
- Carcinoma with melanotic features

Invasive lobular carcinoma

Tubular carcinoma

Invasive cribriform carcinoma

Medullary carcinoma

Mucinous carcinoma and other tumors with abundant mucin

- Mucinous carcinoma
- Cystadenocarcinoma and columnar cell mucinous carcinoma
- Signet ring carcinoma

Neuroendocrine tumors

- Solid neuroendocrine carcinoma
- Atypical carcinoid tumor
- Small cell/oat cell carcinoma
- Large cell neuroendocrine carcinoma

Invasive papillary carcinoma

Invasive micropapillary carcinoma

Apocrine carcinoma

Metaplastic carcinoma

- Pure epithelial metaplastic carcinoma
- Squamous cell carcinoma
- Adeno carcinoma with spindle cell metaplasia
- Adenosquamous carcinoma
- Mucoepidermoid carcinoma
- Mixed epithelial/mesenchymal metaplastic carcinoma

Lipid rich carcinoma

Secretory carcinoma

Oncocytic carcinoma

Adenoid cystic carcinoma

Acinic cell carcinoma

Glycogen rich clear cell carcinoma

Sebaceous carcinoma

Inflammatory carcinoma

Lobular neoplasia

- Lobular carcinoma in situ

Intraductal proliferative lesions

- Usual ductal hyperplasia
- Flat epithelial atypia

- Atypical ductal hyperplasia
- Ductal carcinoma on situ

Microinvasive carcinoma

Intraductal papillary neoplasm

- Central papilloma
- Peripheral papilloma
- Atypical papilloma
- Intraductal papillary carcinoma
- Intracystic papillary carcinoma

Adenosis including variants

- Sclerosing Adenosis
- Apocrine Adenosis
- Blunt duct Adenosis
- Microglandular Adenosis
- Adenomyoepithelial Adenosis

Radial scar/complex sclerosing lesion

Adenomas

- Tubular Adenoma
- Lactating Adenoma
- Apocrine Adenoma
- Pleomorphic Adenoma
- Ductal Adenoma

Myoepithelial lesions

- Myoepitheliosis
- Adenomyoepithelial adenosis
- Adenomyoepithelioma
- Malignant myoepithelioma

Mesenchymal tumors

- Hemangioma
- Angiomatosis
- Hemangiopericytoma
- Pseudoangiomatous stromal hyperplasia
- Myofibroblastoma
- Fibromatosis
- Inflammatory myofibroblastic tumor
- Lipoma
- Angiolipoma
- Granular cell tumor
- Neurofibroma
- Schwannoma
- Angiosarcoma
- Liposarcoma
- Rhabdomyosarcoma
- Osteosarcoma
- Leiomyoma

Leiomyosarcoma

Fibroepithelial tumors

Fibroadenoma

Phyllodes tumor

- Benign
- Borderline
- Malignant

Periductal stromal sarcoma,low grade

Mammary hamartoma

Tumors of the nipple

Nipple adenoma

Syringomatous adenoma

Pagets disease of nipple

Malignant lymphoma

Diffuse large B cell lymphoma

Burkitt’s lymphoma

Extranodal marginal zone B Cell lymphoma

Follicular lymphoma

Metastatic tumors

Tumors of the male breast

Gynaecomastia , Carcinoma :invasive, insitu

ANNEXURE-III

ஆராய்ச்சி தகவல் தாள்

திருநெல்வேலி அரசு பொது மருத்துவமனைக்கு மார்பக கட்டியுடன் வரும் நோயாளிகளுக்கு மார்பக புற்று நோய் உள்ளதா என்பதை கண்டறியும் நவீன பரிசோதனை பற்றிய ஆராய்ச்சி நடைபெற்று வருகிறது.

மார்பக கட்டியுடன் வரும் நோயாளிகளுக்கு மார்பக புற்று நோய் உள்ளதா என்பதை கண்டறியும் நவீன பரிசோதனை எந்த அளவிற்கு பயனளிக்கும் என்பதை அறிந்து கொள்வதே இந்த ஆராய்ச்சியின் நோக்கமாகும்.

நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க நாங்கள் விரும்புகிறோம். முடிவுகளை அல்லது கருத்துக்களை வெளியிடும் போதோ அல்லது ஆராய்சியின் போதோ தங்களது பெயரையோ அல்லது அடையாளங்களையோ வெளியிடமாட்டோம் என்பதையும் தெரிவித்து கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் இருக்கிறது மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியிலிருந்து பின்வாங்கலாம் என்பதையும் தெரிவித்துக்கொள்கிறோம்.

இந்த சிறப்பு பரிசோதனைகளின் முடிவுகளை ஆராய்ச்சியின் போதோ அல்லது ஆராய்ச்சியின் முடிவிலோ தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்துக் கொள்கிறோம்.

ஆராய்ச்சியாளர் கையொப்பம் பங்கேற்பாளர் கையொப்பம்

தேதி:

ஆராய்ச்சி ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு: மார்பக கட்டியுடன் வரும் நோயாளிகளுக்கு மார்பக புற்று நோய் உள்ளதா என்பதை கண்டறியும் நவீன பரிசோதனை பற்றிய ஆராய்ச்சி.

பெயர்:

வயது:

பால்:

தேதி:

உள்நோயாளி எண்:

ஆராய்ச்சி சேர்க்கை எண்:

இந்த ஆராய்ச்சியின் விவரங்களும் அதன் நோக்கங்களும் முழுமையாக எனக்கு தெளிவாக விளக்கப்பட்டது. எனக்கு விளக்கப்பட்ட விஷயங்களை நான் புரிந்து கொண்டு எனது சம்மதத்தை தெரிவிக்கிறேன்.

இதற்கு தேவையான பரிசோதனைகளுக்கு நான் மனமார சம்மதிக்கிறேன்.

கையொப்பம்

ANNEXURE-IV

KEY TO MASTER CHART

1. MENOPAUSE-

   YES-ATTAINED MENOPAUSE

   NO-NOT ATTAINED MENOPAUSE

2. SKIN INVOLVEMENT

   YES-PRESENT

   NO-ABSENT

3. AXILLARY NODE INVOLVEMENT

   YES-PRESENT

   NO-ABSENT

4. GRADING

   1-GRADE 1

   2-GRADE 2

   3-GRADE 3

5. CELLULARITY

   1-POOR

   2-ADEQUATE

   3-GOOD

6. BACK GROUND BLOOD DEBRIS

   1-ZERO

   2-OCCASIONAL

   3-ABUNDANT

7. CELL ARCHITECTURE

NR-NOT RECOGNISED

MR-MODERATELY RECOGNISED

WR-WELL RECOGNISED

8. IMMUNOCYTOCHEMISTRY

NEG-NEGATIVE

POSITIVE-POSITIVE

MASTER CHART

										GRADING OF LESION	CELLULARITY		BACKGROUND BLOOD DEBRIS		MONOLAYERS		CELL ARCHITECTURE		CYTOPLASMIC AND NUCLEAR DETAILS		ICC(FNAC)			I CC(LBC)		
S.NO	NAME	PATH NO	IP NO	AGE	SEX	MENOPAUSE	TUMOR SIZE(cm)	SKIN INVOLVEMENT	AXILLARY NODE	FNAC	FNAC	LBC	FNAC	LBC	FNAC	LBC	FNAC	LBC	FNAC	LBC	ER STATUS	PR STATUS	HER2 /neu	ER STATUS	PR	Her-2/neu
1	PETCHIYAMMAL	F-3696/14	217963	55	F	Y	3	NO	NO	2	2	1	2	2	absent	occasional	NR	MR	good	poor	NEG	NEG	NEG	NEG	NEG	NEG
2	PACKIYATHAI	F-1829/15	10037	50	F	N	3.5	NO	YES	3	2	1	1	1	occasional	absent	MR	NR	poor	good	NEG	POSITIVEP	POSITIVE	NEG	NEG	NEG
3	ANNAL SELVI	F-3905/15	69816	65	F	Y	5	NO	YES	2	1	2	3	2	occasional	occasional	MR	MR	good	excellent	POSITIVE	NEG	POSITIVE	NEG	NEG	NEG
4	VALLIAMMAL	F-3988/14	69884	65	F	Y	7	NO	YES	3	1	2	2	2	absent	good	NR	MR	good	good	NEG	NEG	NEG	NEG	NEG	NEG
5	ANTHONYAMMAL	F-3947/15	71315	60	F	Y	5	YES	YES	1	1	2	3	1	occasional	occasional	WR	WR	good	good	POSITIVEN	NEG	NEG	NEG	NEG	NEG
6	MARIAMMAL	F-3900/14	241066	40	F	N	4	NO	NO	1	1	1	3	1	good	good	WR	MR	excellent	good	NEG	POSITIVE	NEG	NEG	NEG	NEG
7	PUTHIYAL	F-18/15	72956	65	F	Y	8	YES	YES	3	2	2	1	1	good	occasional	MR	WR	good	poor	NEG	NEG	NEG	NEG	NEG	NEG
8	THANGAMMAL	F-147/15	13711	70	F	Y	6	NO	NO	2	2	3	2	3	occasional	good	WR	WR	excellent	good	POSITIVE	POSITIVE	NEG	NEG	NEG	NEG
9	ANNAL	F-155/15	12637	62	F	Y	5	YES	YES	2	2	3	3	1	good	occasional	MR	MR	good	good	NEG	NEG	POSITIVE	NEG	NEG	NEG
10	MARIAMMAL	F-163/15	4332	40	F	N	5	YES	NO	2	2	3	3	1	occasional	good	WR	MR	poor	excellent	POSITIVE	NEG	POSITIVE	NEG	NEG	NEG
11	SANTHI	F-58/15	1013	36	F	N	3.5	NO	NO	1	2	3	3	2	absent	absent	MR	NR	good	good	POSITIVE	POSITIVE	NEG	NEG	NEG	NEG
12	SHYAMALA	F-13/15	4025/15	67	F	Y	6	YES	YES	1	2	3	1	1	good	good	WR	NR	good	excellent	POSITIVE N	NEG	NEG	NEG	NEG	NEG
13	THAMARAISELVI	F-228/15	19005	48	F	N	5	NO	YES	2	2	2	3	1	occasional	good	MR	MR	excellent	good	POSITIVE	NEG	POSITIVE	NEG	NEG	NEG
14	CHELLAMMAL	F-185/15	4653	63	F	Y	4	NO	YES	3	2	1	3	2	good	occasional	WR	MR	good	good	NEG	NEG	NEG	NEG	NEG	NEG
15	SANTHANALAKSHMI	F-349/15	24864	63	F	Y	4.5	NO	YES	3	2	2	2	1	occasional	good	MR	MR	good	excellent	NEG	NEG	NEG	NEG	NEG	NEG
16	NATCHIYAR	F-4083/14	72497	60	F	Y	6	NO	NO	2	1	2	3	2	good	absent	NR	NR	good	good	NEG	NEG	NEG	NEG	NEG	NEG
17	SUBBUKUTTI	F-435/15	950	45	F	N	5	NO	NO	1	2	2	2	1	good	occasional	WR	WR	good	poor	NEG	NEG	POSITIVE	NEG	NEG	NEG
18	SUDHA	F-528/15	10229	48	F	N	5.5	NO	YES	2	2	3	3	2	absent	good	MR	NR	poor	good	NEG	NEGATIVE	NEG	NEG	NEG	NEG
19	ARUMUGAM	F-510/15	25765	55	F	Y	6	YES	YES	1	3	2	2	3	good	occasional	WR	NR	excellent	good	POSITIVE P	POSITIVE	NEG	NEG	NEG	NEG
20	MALLIKA	F-574/15	69785	60	F	Y	5.5	NO	NO	2	2	2	3	2	occasional	good	WR	MR	good	poor	POSITIVE	NEGATIVE	POSITIVE	NEG	NEG	NEG
21	CHANDRA	F-1098/15	64652	55	F	Y	4.5	YES	NO	3	2	2	3	2	absent	good	WR	MR	excellent	good	NEG	NEG	NEG	NEG	NEG	NEG
22	PAPPA	F-1102/15	64835	55	F	Y	4	NO	YES	1	2	2	3	2	good	occasional	MR	MR	good	good	POSITIVE	POSITIVE	NEG	NEG	NEG	NEG
23	IYYAMMAL	F-1187/15	21947	45	F	N	5.5	NO	NO	2	2	2	3	2	occasional	absent	WR	MR	good	excellent	NEG	NEG	NEG	NEG	NEG	NEG
24	ANANDHI	F-1820/15	96795	49	F	N	4	NO	YES	2	2	2	2	2	absent	good	MR	MR	good	good	NEG	NEG	POSITIVE	NEG	NEG	NEG
25	CHELLATHAI	F-1971/15	107115	62	F	Y	5	YES	NO	2	3	2	2	2	occasional	good	WR	WR	excellent	good	NEG	NEG	POSITIVE	NEG	NEG	NEG
26	SEETHA	F-3805/14	233422	40	F	N	3.5	NO	NO	1	1	2	3	1	absent	occasional	MR	WR	good	excellent	POSITIVE N	NEG	POSITIVE	NEG	NEG	NEG
27	CHELLAMMAL	F-601/15	38006	51	F	Y	3	NO	NO	1	1	2	1	3	good	good	NR	NR	excellent	poor	POSITIVE	POSITIVE	NEG	NEG	NEG	NEG
28	CHIDAMBARAM	F-53/15	4711	75	F	Y	8	NO	YES	3	2	2	3	1	occasional	good	WR	WR	good	good	NEG	NEG	NEG	NEG	NEG	NEG
29	SALIKA BEEVI	F-503/15	34558	50	F	Y	6	NO	NO	2	2	2	2	2	absent	occasional	MR	WR	good	excellent	NEG	NEG	NEG	NEG	NEG	NEG
30	VIJAYALAKSHMI	F-535/15	101514	45	F	Y	4.5	NO	NO	1	2	2	2	2	occasional	good	MR	MR	good	good	POSITIVE	NEG	NEG	NEG	NEG	NEG